Towards the optimization of canola meal as a protein source for Japanese quails using exogenous feed enzymes

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DECLARATION

I, Caven Mguvane Mnisi, uphold that:

i. This thesis hereby submitted by me for the degree of Doctor of Philosophy in Agriculture in Animal Science at the North-West University is my original research work.

ii. This thesis is not submitted for any degree or examination at any university other than the North-West University.

iii. The use of information and materials from any other source has been properly and fully acknowledged.

iv. Reported results were generated by me and not by any other scholar or organisation.

v. Ethical considerations (NWU-00521-16-A9) have been approved by the Animal Research Ethics Committee, North-West University (AREC-MC) as the study involved the rearing and slaughter of quails. The welfare of the Japanese quails complied with the guidelines for the care and use of research animals (South African Bureau of Standards, 2008).

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GENERAL ABSTRACT

Broadly, this study was an attempt to valorise canola meal as a source of protein for Japanese quails (*Coturnix coturnix japonica*) in place of soybean meal using feed enzymes. Growth performance, haematology, serum biochemistry, carcass characteristics and meat quality of the quails were used as indicators in three experiments. The strategy of choice was the use of feed enzymes; a carbohydrase multi-enzyme (*endo*-1.4-beta-xylanase (> 1–< 3%; 5600 TXU/g, EC no: 232-800-2) and *endo*-1.4-beta-glucanase (> 0.3–< 1%; 2500 TGU/g, EC no. 232-734-4)) and a protease (75'000 PROT/g; EC/IUB no. 3.4.21) mono-enzyme to enhance utilization of CM-based diets. The first objective was to establish the maximum tolerance level of quails for CM without feed enzyme treatment. For four weeks, quails were fed five experimental diets formulated as follows: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal. Quails fed diet CM175 had the lowest (P <0.05) feed intake whereas no differences were observed among the other four treatment groups. There were no dietary effects on average weight gain (AWG), gain: feed ratio (GFR) and haematological parameters of quails. All serum biochemical parameters, except for alkaline phosphatase (ALP), were not influenced by experimental diets. Quails on CM25 had higher ALP (161.0 U/L) than those on CON diet (37.3 U/L). Carcass characteristics and dressing percentage of quails across diets were also observed to be similar. Diets influenced the length of small intestines with quails fed diets CON and CM50 having the longest small intestines, which did not differ (P >0.05). No dietary effects were observed in meat quality parameters immediately and 24 h post slaughter, except for meat chroma

measured 24 h post slaughter. Quails fed diet CM25 had the highest chroma (7.39) while those on diet CM125 had the lowest (3.58). It was, therefore, established that CM can replace SBM in quail diets up to 12.5% without compromising the birds' growth performance, health and quality of meat. The highest inclusion level of canola (CM175) reduced feed intake, which could be a result of higher levels of fibre and non-starch polysaccharides in the diet. It was hypothesized that the use of feed additives such as enzymes may improve the utilization of CM in quails allowing its inclusion at levels higher than 12.5%. Another trial was, therefore, designed to investigate the potential to enhance the utilization of diets containing CM beyond the 12.5% level tolerated by quails through the use of a dietary carbohydrase multi-enzyme. Thus, the effect of including a carbohydrase multi-enzyme in canola-based quail diets on growth performance, haemo-biochemical parameters, carcass characteristics and meat quality traits was investigated. The application of this multi-enzyme was aimed to improve the utilisation of canola by breaking down the presence of non-starch polysaccharides (NSP) such as glucans and xylans that are known to interfere with digestion and negatively affect feed intake. In this study, CM was only included at 17.5%, a level higher than the maximum tolerable inclusion rate (12.5 %) established in experiment one. For three weeks, quails were fed five dietary treatments formulated as follows: CON = control diet (a commercial growers diet with no CM inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, and CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, 10% or 15% (CM50, CM100 and CM150, respectively). There was a significant diet × week interaction on weekly feed intake indicating that the effect of the diet changed as the quails matured. In both weeks 8 and 9, feed intake showed significant differences between diets. Diets had no influence on haematology and serum biochemical parameters of Japanese quails. Adding the carbohydrase multi-enzyme had no significant

effect on internal organs, carcass and meat quality of quails. It was, therefore, concluded that the carbohydrase multi-enzyme treatment does not improve the utilisation of a CM-based quail diet. As another attempt to improve the utilisation of CM, the potential of a protease mono-enzyme treatment of canola-based diets to enhance growth performance, haemo-biochemical parameters, carcass characteristics and meat quality parameters in Japanese quails was investigated. For four weeks, quails were offered 5 dietary treatments formulated as follows: CON = control diet (a commercial growers mash with no CM inclusion), CM0 = control diet in which 17.5% of SBM was replaced with CM, and CM0 diet treated with 10, 20 and 30% of protease enzyme (CM10, CM20 and CM30, respectively). Protease inclusion had no significant effect on feed intake, weight gain, GFR, haemo-biochemical parameters, internal organs, carcass characteristics, and meat quality traits. It was, therefore, clear that the inclusion of protease feed enzyme did not enhance the value of CM as a protein source in Japanese quail diets. The inclusion of either a carbohydrase multi-enzyme or a protease mono-enzyme did not improve the utilisation of a CM-based quail diet. It is, therefore, recommended that the inclusion rate for canola meal as a replacement for SBM in Japanese quail diets be capped at 12.5% and that there is no benefit in applying feed enzymes where higher CM inclusion levels are used.

Keywords: Canola meal, Exogenous enzymes, Haemo-biochemistry, Japanese quails, Meat quality, Soybean meal

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I am grateful to my family for their love, support and encouragement throughout this journey.

DEDICATION

I dedicate this thesis, in its entirety, to my family - boMvuleni!

"It isn't the mountains ahead to climb that wear you out; it's the pebble in your shoe"

Muhammad Ali

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ii. **C. M. Mnisi**, V. Mlambo, K.G.G. Phatudi and T.B. Matshogo., 2017. Exogenous carbohydrases do not improve nutritional status, growth performance, and meat quality traits of female Japanese quails fed canola-based diets. *South African Journal of Animal Science, volume 47, issue 6, pages: 923 – 932.* **(Published).**

iii. **C. M. Mnisi** & V. Mlambo., 2018. Protease-treated canola-based Japanese quail diets: Effect on physiological parameters and meat quality traits. *Animal Nutrition.* **(Submitted)**.

iv. **C. M. Mnisi** & V. Mlambo., 2018. Towards food and nutrition security in semi-arid regions of South Africa: The potential role of quail production. *South African Journal of Animal Science.* **(Under preparation).**

LIST OF ABBREVIATIONS

AA	:	Amino acids
ALP	:	Alanine phosphatase
ALT	:	Alanine transaminase
ANF	:	Antinutritional factors
AWFI	:	Average weekly feed intake
AWG	:	Average weight gain
CCK	:	Cholecystokinin
CCW	:	Cold carcass weight
CM	:	Canola meal
CP	:	Crude protein
CT	:	Condensed tannins
DM	:	Dry matter
EC	:	Enzyme commission
FI	:	Feed intake
GFR	:	Gain: feed ratio
GLM	:	General linear model
H	:	Hour
Kg	:	Kilogram
LSMEANS	:	Least square means
MCH	:	Mean corpuscular haemoglobin
MCHC	:	Mean corpuscular haemoglobin concentration
MCV	:	Mean corpuscular volume
NIRs	:	Near infrared reflectance spectroscopy
NS	:	Not significant

NSP	:	Non-starch polysaccharides
OM	:	Organic matter
PI	:	Protease inhibitors
SAS	:	Statistical analyses system
SBM	:	Soybean meal
SEM	:	Standard error of the mean
TI	:	Trypsin inhibitors
WCW	:	Warm carcass weight

1 CHAPTER ONE - GENERAL INTRODUCTION

1.1 Background

The Japanese quail (*Coturnix coturnix japonica*) is the smallest avian species that is commercially farmed for meat and egg production. This bird has received worldwide recognition not only as a laboratory animal but also as a source of protein for human consumption, particularly for the poor and the landless citizens (Panda & Singh, 1990; Baumgartner, 2007). The Japanese quail is a fairly recent entrant into the poultry industry around the world. Prior to this development, quails were simply considered as wild birds of little commercial significance. However, observations from domestication trials reveal favourable qualities such as fast growth rates, tolerance to harsh nutritional conditions, resistance to numerous avian diseases, short generation intervals and early (6 weeks of age) sexual maturity (Randall & Bolla, 2008; Mnisi *et al.*, 2017). These attributes have prompted renewed efforts to improve their production and contribution to household food security and ensure sufficient nutrition for the fast-growing human population (Wickramasuriya *et al.*, 2015). The leading challenge in profitable and sustainable quail production is the growing competition for food resources between humans and animals. This is because some of the feed ingredients used during animal feed formulation are also direct food resources for humans. For example, soybean (*Glycine max*) is one of the major protein sources used in the poultry industry and is also food for human beings. It is well-documented that soybean is an excellent protein source due to a well-balanced and readily digestible amino acid profile (Newkirk, 2010; Beski *et al.*, 2015). Due to increased demand from the biofuel industry, food and feed sectors (Barekatain *et al.*, 2015), the competition for soybean has resulted in an increase in its market price worldwide (Newkirk, 2010). Exploring inexpensive and readily available protein sources for quail

farming is necessary for the continued sustainable growth of the industry. One such alternative is canola (*Brassica napus*) meal, a by-product generated after industrial extraction of oil from canola seeds. The seeds contain about 40–42% of oil that can be used for both human consumption and biodiesel production (Unger, 1990). Unlike soybean, there is no competition with humans for canola meal (CM) and thus the demand and, consequently, market price are low (Unger, 1990; Bell, 1993; Canola Council of Canada, 2009). Canola meal is a potential protein source for animal feed, with an amino acid profile that is comparable to that of soybean (Sariçiçek *et al.*, 2005; Aidera & Barbanab, 2011; Barekatain *et al.*, 2015; Wickramasuriya *et al.*, 2015). The utilisation of CM, particularly in the poultry industry, has been restricted by the presence of undesirable plant secondary compounds such as polyphenolics, phytates, non-starch polysaccharides (NSP), erucic acid, glucosinolates (Ghodsvali *et al.*, 2005; Canola Council of Canada, 2009; Chen *et al.*, 2015), and trypsin/protease inhibitors (Berot *et al.*, 2005; Hussain, 2015). These antinutritional factors (ANF) and high dietary fibre content in canola meal may reduce animal performance and compromise the quail's health status (Wickramasuriya *et al.*, 2015). Nonetheless, genetic manipulation of canola varieties through plant breeding have resulted in the development of canola cultivars with low erucic acid (< 2%) and glucosinolate (< 30 μmol/g) levels, reduced concentration of fibre and higher concentration of crude protein and oil compared to the conventional canola (Jia *et al.*, 2012; Berrocoso *et al.*, 2015; Parr *et al.*, 2015). However, it is widely accepted that these efforts have not completely eradicated these antinutrients resulting in canola meal playing second fiddle to SBM as a source of dietary protein in the poultry industry.

1.2 Problem statement

Soybean meal has been widely used as an excellent protein source in the diets of simple non-ruminants for many years. As already stated, the quality of SBM as a potential feed ingredient is unquestionable and needs no further emphasis (Beski *et al.*, 2015). High market prices of SBM have, however negatively affected the viability of several avian businesses, with farmers failing to cope with increased feed costs. Recently, the possibility of replacing soybean with canola in poultry diets has emerged as a possible avenue through which profitability of avian enterprises can be enhanced (Sariçiçek *et al.*, 2005). The use of CM as a potential protein source is essential to reduce feed costs (Barekatain *et al.*, 2015). According to Nowlin (1991), canola is a relatively inexpensive winter crop that has high protein content (36–39%), which indicates its potential to meet the quail's protein requirements. Scanes *et al.* (2004) argued that the usefulness of a protein source for poultry depends on its ability to provide sufficient amount of digestible essential amino acids as well as low levels of antinutritional compounds. Although canola has a reasonably well-balanced amino acid profile (Wickramasuriya *et al.*, 2015), it contains secondary plant compounds (Li *et al.*, 2015), which could be detrimental to the growth performance as well as health of quails. The presence of polyphenolics, phytate, protease/trypsin inhibitors and NSP in CM reduce nutrient utilisation and bioavailability thus negatively affecting growth performance (Wickramasuriya *et al.*, 2015). The NSP in CM are not susceptible to digestion by endogenously-produced digestive enzymes, while trypsin inhibitors interfere with the function of pepsin and trypsin (Berot *et al.*, 2005; Hussain, 2015; Wickramasuriya *et al.*, 2015). In addition, inclusion levels of CM greater than 20% in poultry diets adversely affected the birds' performance (Meng *et al.*, 2006; Payvastagan *et al.*, 2012). Canola meal has high fibre components (294.0 g/kg neutral detergent fibre; 219.0 g/kg NSP; and 107.0 g/kg lignin), which limit the value of canola as a protein

source in quail feed formulations (Khajali & Slominski, 2012). Because of all the limitations of canola, it is imperative that strategies be sought to enhance the feed value of CM if it is to be used as an alternative for SBM, the gold standard of protein sources in animal diets.

1.3 Justification

Low nutrient utilisation and poor animal performance at higher canola inclusion levels can be attributed to the presence of ANF in the CM. In an attempt to find solutions for the poorer performance and lower nutrient digestibility encountered when canola-based diets are offered to birds, Shen *et al.* (1983) found that steam-pelleting improves nutrient availability allowing for the dietary inclusion of up to 20% CM without altering the birds' performance. Salmon *et al.* (1988) reported that heat-treatment of CM also enhanced nutrient utilisation. Meng *et al.* (2006), reported that grinding disrupts the cell wall and increases the exposure of nutrients to digestive enzymes. However, Barekatain *et al.* (2015), who investigated the effect of grinding and pelleting conditions of canola seed on bird performance and nutrient utilisation, reported that regardless of the processing conditions, inclusion levels of CM greater than 150 g/kg reduce feed intake and weight gain. Autoclaving CM increased neutral detergent fibre and acid detergent insoluble nitrogen and subsequently reduced amino acids digestibility (Almeida *et al.*, 2014). Other studies have focused on the use of exogenous enzymes to improve the feed value of canola. For example, Sariçiçek *et al.* (2005) treated CM with exogenous phytases and carbohydrases to determine the performance of growing and laying quails and reported no enzyme influence on egg parameters. With close to 60% of the phosphorus being in phytate form in CM (Adewole *et al.*, 2016), several feed manufacturing companies have included exogenous phytase during feed formulations to enhance phosphorus bioavailability (Selle & Ravindran, 2007). There is, therefore, evidence that the use of

enzyme-treated poultry diets can be a way to promote intensive production on a large-scale (Khajali & Slominski, 2012; Singh *et al.*, 2017). Romero *et al.* (2013) together with Cowieson and Roos (2016) argued that exogenous enzymes such as carbohydrases and proteases improve the utilisation of CM and increase broilers' performance. Although there are several candidate strategies that may be used to optimize the feed value of CM for Japanese quails, the use of enzymes seems to have produced more consistent positive outcomes because they complement endogenous digestive enzymes produced by birds. Furthermore, exogenous enzymes are now largely produced by several feed manufacturing companies, which means that they are readily accessible to quail producers. To our knowledge, no studies have attempted to establish the tolerance level of quails to CM and there are no recommended inclusion levels of carbohydrase and protease for canola-based Japanese quail diets. The study, therefore, seeks to establish the tolerance level of quails to graded levels of canola-based diets and to improve growth performance, haematological and serum biochemical parameters, carcass characteristics, and meat quality traits of female Japanese quails of canola-based diets through the application of a carbohydrase multi-enzyme (*endo*-1.4-beta-xylanase: 5600 TXU/g and *endo*-1.4-beta-glucanase: 2500 TGU/g) and a protease mono-enzyme (75'000 PROT/g; EC/IUB no. 3.4.21).

1.4 Objectives

The study is designed to optimize canola meal as a source of protein for female Japanese quails (*Coturnix coturnix japonica*) in place of soybean meal using feed enzymes. The initial experiment was designed to establish the maximum tolerance level of quails for CM when used as a partial replacement for SBM. Further experiments were designed to investigate the potential to enhance the utilization of CM-based diets and enable the inclusion of CM at higher levels through the use of feed enzymes. For these subsequent experiments, CM was included at a level higher than the maximum tolerable inclusion rate

established in the first experiment. Thus, the following specific objectives guided the study:

a. To determine the growth performance, haematological and serum biochemical parameters, carcass and meat quality characteristics of female Japanese quails to graded levels of CM in place of SBM
b. To determine the effectiveness of a carbohydrase multi-enzyme and a protease mono-enzyme to enhance the utilization of canola-based diets as measured by several physiological and meat quality response parameters in female Japanese quails.

1.5 Hypotheses

a. The null hypothesis of the initial experiment was that canola-based diets promote similar performance, in terms of growth performance, blood parameters, carcass characteristics and meat quality traits in female Japanese quails, to the soybean-based positive control diet.
b. The alternative hypothesis of the subsequent experiments tested whether there are differences between enzyme-treated CM and untreated CM in terms of growth performance, haematology, serum biochemistry, carcass characteristics and meat quality traits of female Japanese quails.

1.6 Summary

Quail farming can be a reliable source of dietary protein for human consumption and has the potential to alleviate issues of food nutrition insecurity in semi-arid regions. Finding alternative feed ingredients such as canola meal to replace the gold standard protein source, soybean meal, is a strategic way to reduce feed costs. However, canola meal has antinutritional factors which reduce its utilisation especially in the poultry industry. Therefore, the use of feed enzymes such as carbohydrases and proteases can improve the utilisation of canola in Japanese quail-based diets.

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2 CHAPTER TWO - LITERATURE REVIEW

2.1 Introduction

Over more than one hundred years, the poultry industry has evolved from backyard farming units into a complex and highly integrated industry. Currently, it is one of the largest agricultural sectors in South Africa contributing more than 16% of agricultural gross domestic product (Bolton, 2015). The industry plays a pivotal role in creating direct and indirect job opportunities for about 108 000 people throughout its value chain and related industries (Bolton, 2015). It supports many large and small-scale enterprises and also provides a strong platform for rural development, as well as food security programmes. It is recognised as the largest supplier of high quality protein (30%) for human consumption with a per capita of 48.85 kg of total poultry products (FAO, 2012).

The human population benefits greatly from poultry meat and eggs, which provide food containing high-quality protein, and low levels of fat with a desirable fatty acid profile (FAO, 2009). In developing countries, poultry products are widely accessible and relatively inexpensive and they are necessary to help meet shortfalls in essential nutrients for impoverished people. Ravindran (2013a), reported that several incidences of metabolic diseases associated with deficiencies in critical dietary nutrients in humans can be reduced by the consumption of poultry products (meat and eggs), which are rich in all essential nutrients, with the exception of vitamin C. The United Nations reports that the current global human population of more than 7 billion is expected to grow to 8.4 billion by mid-2030 and 9.6 billion by mid-2050 (United Nations, 2017). This rapidly increasing human population increases pressure on the demand for high quality foods in general, and white meat in particular. For this reason, improving and intensifying quail farming is necessary to meet the demand for poultry products.

In South Africa, poultry products have the largest consumption rate compared to other meat products and this can be strongly attributed to the fact that they are inexpensive compared to beef, chevon, mutton and pork products (Delany, 2003). The high cost of poultry production needs to be addressed since it is the major challenge for poultry producers. It is difficult for poultry farmers to meet the high demand of poultry products due to uncontrollable avian diseases, high mortality rates, low rainfall and droughts, high cost of energy and labour, poor infrastructure and lack of technical expertise. Indeed, high feed cost, which accounts for more than 70% of total costs of production, has been the driver of the efforts to identify alternative feed ingredients for least-cost and effective poultry production (Wickramasuriya *et al.*, 2015).

Soybean meal (SBM) is the major source of protein for poultry diets. Due to the high demand of soybean, its market prices have increased and as a result it is now unaffordable to emerging farmers. Even though the demand is high, the production of soybean is declining due to unfavourable climatic conditions such as low rainfalls and droughts. This, therefore, calls for an urgent search for alternative protein sources that are readily available and inexpensive such as canola meal (CM). The use of CM has been limited because of low available protein and energy content when compared to SBM. Soybean meal has been universally used as a standard or reference plant protein source in the animal industry. However, CM could be a suitable feed ingredient in poultry feeds but its inclusion rate needs further investigation to ensure safe and beneficial utilisation without compromising the birds' health (Campbell & Smith, 1979).

Inclusion of canola beyond 30% have detrimental effects on quails' growth performance and health, this might be due to the high fibre content, trypsin inhibitors and non-starch polysaccharides (NSP), which reduce digestibility and nutrient bioavailability (Bell, 1993;

Hussain, 2015). The use of exogenous enzyme supplementation to fibrous and NSP-rich diets improves nutrient digestion and absorption by partially hydrolysing NSP and reducing the viscosity of gut contents (Almirall *et al.*, 1995). Indeed, Slominski and Campbell (1990) have shown that the application of cell wall-degrading enzymes improves the digestibility of canola polysaccharides in poultry.

2.2 The Japanese quail strain

There are diverse breeds of quails, with over 100 of them mostly in found in Asia and North-America. These breeds are divided into two main groups: Old World quail and New World quail (OMLET, 2004). The Japanese quail, *Coturnix coturnix japonica*, is a species of the Old-World quail found in East Asia that belongs to the order *Galliformes* and the family *Phasianidae* (Minvielle, 2004). *Coturnix coturnix japonica* is a small, ground nesting wild bird that spends most of the time scratching and digging up food from the ground. Quails are fairly round in shape with females characterised by light tan feathers together with black speckling on the throat and upper breast, whereas the males have rusty brown throat and breast feathers (Minvielle, 2004). These birds reach maturity in about six weeks of age and the females start laying eggs around 50 days of age (Randall & Bolla, 2008). Where proper care and management is offered, the hens can lay 200 eggs in their first year of lay (Ayaşan, 2013). Adult males have a cloacal gland that is used for reproductive fitness evaluation (Randall & Bolla, 2008). This bulbous cloacal gland is located on the upper edge of the vent and is responsible for the secretion of a white, foamy material, which is thought to seal the semen after mating in the females.

Provision of light 14 to 18 hours per day is necessary to maintain high fertility and maximum egg production, suggesting that for high egg production to be achieved supplementary lighting should be rendered (Randall & Bolla, 2008). For optimum

production, environmental conditions should be adjusted according to the age of the quails. Quails and their eggs are food to various natural predators mainly because of their small body sizes and their nutritious eggs (Randall & Bolla, 2008). Even human beings tend to be predators of wild quails, although a majority now prefer those that have been reared under intensive systems.

Table 2.1. Characteristics of Japanese quails

Characteristic	Japanese quails
Body weight	Adult females = 160 – 250 g
	Adult males = 100 – 180 g
	Chicks = 6 – 8 g
Average egg weight	10 g
Egg colour	Mottled brown, covered with a light blue, chalky material
Egg incubation	17 – 21 days
Life span	3 - 5 years

Adapted from Randall and Bolla (2008)

2.3 Evolution of quails in the poultry industry

The poultry industry is largely dominated by commercial broiler and layer production, with a few indigenous chickens and other birds such as ostrich, ducks and turkey. Expansion of the poultry industry is necessary to maintain continuous supply of meat and egg products for human consumption (Ayaşan, 2013). A feasible species for this expansion is the quail. The quail sector has been one of the largest and fastest growing

agro-industries throughout the world, with many people rearing quails for commercial purposes (Puspamitra *et al.*, 2014). In recent years, the production of Japanese quails has considerably increased primarily due to their desirable genetic potential, which plays a pivotal role in ensuring food nutrition and security. Other poultry birds can be raised along with quails for the production of eggs and meat (OMLET, 2004).

The ability of quails to reach market weight earlier means that quail producers do not have to wait for a long time before selling their products. In Japan, France and Spain, the poultry industry is currently dominated by commercial rearing of quails because of their immense abilities to survive various types of climatic and environmental conditions (Gil, 2003; Minvielle, 2004). Amongst the benefits of quails as new entrants to the poultry industry is that their feeding costs are reasonably lower than those of chickens or other domesticated birds, suggesting that quail producers can save enough money on feeding costs, allowing them to sustain their enterprises as they would gain maximum profits at minimum expenditure.

2.4 Quail farming

Quail farming is a portion of the poultry industry that contributes high-quality dietary protein for human consumption. Ali *et al.* (2012) reports that quail farming aims to diversify and strengthen animal protein production in order to close the gap between demand and supply. Many countries are farming quails mainly for household consumption and up-market sales (Siddique & Mandal, 1996; Ali *et al.*, 2012). Quail farming is currently a profitable business to complement chicken, duck and turkey farming. This is because the small body sizes of quails can allow rearing of many quails in a given space, for example six to seven quails can be reared in the same amount of space required by an adult chicken (Ali *et al.*, 2012; Puspamitra *et al.*, 2014). Quail farming also comes with

great benefits such as low labour required together with less capital needed because a small pen or cage can accommodate a bunch of quails. Indeed, Nasar *et al.* (2016) reported that the desirable economic traits of quails are profitable because low capital investments are required as compared to chicken and duck, which have almost the same profit margin. In addition, quails are tolerant to numerous avian diseases thus omitting the cost of vaccinations and treatments, which in turn favour the increasing demand for organic products produced with minimal use of additives (antimicrobial growth promoters) and chemicals (Mnisi *et al.*, 2017).

Quails can survive different environmental conditions, a feature which is acquired from the maternal substances that are deposited into the eggs during laying (Gil, 2003). Opportunities that come with quail farming are that they help create a source of living for emerging farmers who are currently active in increasing the domestic poultry produce through egg and meat production in South Africa. Farming quails do not come as easy as one can elaborate, it also comes with challenges. Often farmers struggle when making a decision on which breed to rear, this is because some breeds of quails are feed wasting and aggressive – injuring other fellow quails and dominating during feeding which consequently affect production. Many factors such as predation, diseases, and parasites affect the farming of quails. Challenges that come with quail farming include sub-optimal nutrition, market inaccessibility, lack of knowledge on quail production and spoilt eggs as a result of cracks, infertility and embryonic mortalities. Nonetheless, quail farming should be encouraged to create employment, extra income and maintain a valuable source of meat and egg (Nasar *et al.*, 2016).

2.4.1 Production systems

The choice of a production system is usually the farmer's preference. Farmers first consider their financial abilities, which primarily determine that production system they can manage. Time and space are also some of the factors that farmers have to consider before they choose a production system, for example the intensive production system needs daily or regular supervision, while the extensive production system requires too much land space for farming (Sonaiya, 2003). Just as in the production of broilers and laying hens, nutrition is one of the most important factors negatively impacting production costs of quail production, primarily due to the continuous fluctuation of prices of traditional dietary ingredients such as soybean meal inclusion, which stimulates increasing interest in possible alternative protein sources (Farahat *et al.*, 2013). Quail farming has evolved through three production systems, which are the traditional, small-scale semi-commercial and large-scale commercial systems (Ravindran, 2013a). These systems are based on a unique set of management styles and technologies. They differ markedly in terms of investment required, type of quails used, husbandry practices and inputs such as feed. The feed resources, feeding methods and feed requirements vary widely depending on the system used.

2.4.1.1 Traditional production system

The traditional system is the most common type of quail production in developing countries. Possible feed resources for the quails reared in this system includes household wastes, materials from the environment (insects, worms, greens and seeds), crop residues, fodders and water plants and industrial by-products. The development of extensive poultry systems is determined by the competition for feed resources. This system is most favourable where biomass is abundant, but in areas with limited natural resources and low

rainfall, the competition for natural resources with other animals can be extreme (Ravindran, 2013a). Extensively reared quails play a major role in ensuring food security in rural communities of most developing countries. This type of farming is usually practised by communal farmers for household support not for sale, it is also recommended for resource-poor farmers because quails are allowed to scavenge for their own feed, with no shelter being offered, and there is no controlled breeding leading to the development of new quail strains.

Quails are omnivorous animals feeding on ants, insects, kitchen leftovers and ground feed. This production system remains the favourite from the public as no drugs and/or medication are used. The development of organic farming, which is similar to the traditional system, had been initiated as a result of public concerns about the usage of antibiotic growth promoters, hormones and vaccination drugs. Most consumers consider traditionally raised quails to contain no drug residues since their rearing management mimics that of organically farmed birds (Sanka & Mbaga, 2015). Any community member can practise this type of farming because it is inexpensive to execute and labour free. Extensive farming addresses the issue of poverty in rural areas by ensuring that each household can decide to have a backyard flock (Sonaiya, 2003). However, under extensive management conditions, Japanese quails rarely attain their full production potential due to exposure to risks that threatens their survival and productivity such as poor infrastructures and/or shelter that expose quails to theft and predators. In addition, uncontrolled breeding may result in disease transmission and inbreeding, leading to genetic defects and other abnormalities.

2.4.1.2 Semi-intensive production system

Semi-intensive production systems are characterized by small to medium flock sizes of 50 to 500 quails confined to a large piece of land but are allowed to roam around and search for food within the confinement. Birds are typically confined overnight and are let out in the morning to scavenge (Ahlers *et al.*, 2009). However, several feeding strategies may be used in this system for example on-farm mixing of complete rations, using commercial and locally available feed ingredients or dilution of local ingredients with purchased feeds or blending of local ingredients with purchased concentrated mixtures. Production can be either for subsistence or for sale. This type of farming system can be practised by small-scale producers because many quails can be reared in a single cage.

Ravindran (2013a) suggested that people with little or no experience of quail farming may invest in smallholder intensive production and build a small quail house near settlements or suburbs. In addition, this system requires low investment and it brings high returns, there is significant savings in feed costs accompanied by quality meat, which is lean and fat free compared to birds grown in intensive production systems. However, growth and egg production are likely to be less when compared to quails reared intensively with better feed resources. This system requires considerable amount of fencing and quails can only mate with the quails within the confinement. The shelter provided is made from various materials, including wood and leaf material from local trees or shrubs. Losses may be encountered due to predation or theft, and failure to locate eggs that are laid in bushy areas, as a result, more labour is required to manage flocks in the semi-intensive system compared to the intensive system (Ravindran, 2013a).

2.4.1.3 Intensive production system

Large-scale commercial production of quails was recorded to have started in the 1920s in Japan followed by the successful introduction of quails in America, Europe, and the Middle East between 1930 and the 1950. This system is the most dominating production system in developed and many developing countries, which is characterized by the incorporation of highly sophisticated production units with high-producing modern quail strains (Ravindran, 2013a).

In this system, quails are reared indoors until slaughter or until the end of their production cycle, which means that quails must receive proper care and good management. On a daily basis, quails must be offered commercial diets, and a lot of labour is required to ensure cleaning, feeding and watering. Farming of Japanese quails on a large scale would rely greatly on high protein and energy feeds derived from soybean and cereals. Feed is therefore the most important variable cost component, accounting to 70% of production costs. High productivity and efficiency depend on feeding nutritionally balanced feeds that are formulated to meet the quails' nutritional requirements.

Randall and Bolla (2008) reported that when proper care is provided, Japanese quails can lay close to 200 eggs in their first year of laying. Chowdhury *et al.* (2006) also stated that productivity and good health of these quails can be doubled with nutritionally balanced diets and management conditions. Many developing countries are now investing heavily on intensive commercial systems of quail production to provide meat and eggs for the growing human populations. Therefore, more research under such system on the productive parameters (body weight, egg production, egg weight) and reproductive parameters (age at sexual maturity, fertility and hatchability) of Japanese quails is required (Faruque *et al.*, 2013).

2.5 Digestion in the quail

Japanese quail is a simple non-ruminant, reflecting that the amount of feed consumed by the quail requires a proper functioning digestive system for efficient break-down of feed. Dingle (1990) reported that the utilisation of nutrients from the diet is a key element in the normal functioning of a bird. The digestive tract comprises of a crop, which is an expansion of the oesophagus located in the lower neck area, a glandular stomach (proventriculus), a muscular stomach (gizzard) and intestines (Bradley, 1960). To achieve high performance in a modern commercial poultry enterprise, quails must be offered high quality diets that consist of easily digested ingredients. Therefore, understanding the digestive system of quails is essential for developing an effective and economical feeding program, in order to take necessary actions if something is wrong (FAO, 2012).

Extensive knowledge on quails' digestive system and how it carries out its digestive and metabolic functions is necessary for effective management and production. Quails acquire energy and other essential nutrients through the digestion of natural feedstuffs, however, minerals, vitamins and essential amino acids such as lysine, methionine, threonine and tryptophan are often offered as synthetic supplements. One major limitation on quails' digestion is the fact that they do not produce enzymes that can break down fibre, suggesting that less fibrous diets should be provided to ensure optimal quail performance. As soon as the quail consumes feed, the feed is thoroughly moistened and mixed with saliva and mucous from the mouth and oesophagus. Endogenous amylase is responsible for the breakdown of complex carbohydrates, particularly starch, is produced by the salivary and oesophageal glands. However, the amount of enzyme action is minimal and the first major enzyme activity takes place in the proventriculus and in the gizzard (Bedford & Cowieson, 2012).

2.5.1 Feed intake and utilisation

Quails have a wide variation of eating habits, for example, they eat meals at 15-minute intervals during daylight hours and, to some extent, during darkness (Bradley, 1960). They eat larger portions at first light and in the late evening. The following factors affect quails feed intake: age and body weight, environmental temperature, energy content of the feed and level of other key nutrients, stage of production, water quality and cleanliness, and the health status of the birds. Similar factors affect the rate of movement of the consumed feed through the digestive system with a meal of normal food taking approximately 4 hours to be digested in young quails, 8 hours in the case of laying hens and 12 hours for broody hens.

Coarser grains take longer to digest than cracked grain, in addition, some whole grain pass through the digestive system unchanged (Bradley, 1960). The pattern of feed intake and its passage through the digestive system are the main factors that influence secretory and hence the digestive activities. This is probably because of the high metabolic rate of the fowl and as a result a constant supply of food is required by the digestive system. This continuous supply is maintained by the crop, which functions as a reservoir for the storage of feed before digestion. The crop consequently permits the fowl to consume its food at periodic meals. There is a wide variability between quails in relation to their eating behaviours, even those in the same flock. Some quails consume small amounts at short intervals, while others eat larger amounts at wider intervals (Gil, 2003).

2.5.2 Digestion of complex feed particles

The utilisation of nutrients from the diet is a fundamental part in the normal functioning of a bird (Bell, 1993). Knowledge on the functioning of the digestive system is necessary for the effective management of the quails, therefore, research on the digestive system and its

processes is an important facet in quail production. The bird produces digestive enzymes that play a significant role in the digestive process of reducing complex feed compounds consumed by the quail into small particles that can be absorbed across the intestinal wall. Feed materials that escape enzyme action along the digestive tract are subjected to microbial breakdown in the lower gut, which provides the digestive system a partial recovery of some nutrients (Bell, 1993). However, quails have limited capacity to utilise fibrous diets, suggesting a need to conduct studies that would define fibre tolerance in quails. Mpofu *et al.* (2016) reported that birds exposed to high dietary fibre tend to have long intestines as an adaptive mechanism to deal with increased amount of fibre. Bell (1993) reported that high fibre content in the canola is responsible for its low energy values and ultimate poor performance. In addition, the NSP (18%) present in the canola have significant influence on feed intake. Quails given a fibrous diet such as canola would initially respond by increasing feeding intake as a way to cater for nutrient dilution and thereafter reduce feed intake because the fibrous substrates compact the crop and other digestive organs, which negatively affect the entire digestive system.

2.5.3 Faecal output

The remaining feed material consists of waste and undigested feed particles are mixed with urine in the cloaca and eliminated from the body as faeces. The form of the faeces varies noticeably, but they are typically round, brown to grey mass topped with a cap of white uric acid from the kidneys (Randall & Bolla, 2008). The contents of the caecum are also discharged periodically as discrete masses of brown, glutinous material. The average daily faecal excretion of laying quail hens ranges from 100 to 150 g. These droppings are composed of roughly 75% water, which air dry under favourable conditions to almost 30% water (Nasar *et al.*, 2016).

2.6 Nutritional requirements of quails

Smith (2005) reported that minimum dietary requirements for essential nutrients in the feed are important to achieve the desired results from the birds. It is important to consider that nutrients required by quails vary according to age and the purpose of production, i.e. whether the quails are kept for meat or egg production. Quails should have access to clean and fresh drinking water at all times because deprivation of water for more than 36 hours may lead to mortality in quails of all ages. However, water intake may be influenced by several factors such as temperature, humidity, salt and protein levels in the diet, the productivity of the quail and also its ability to resorb water in the kidney. Quails require nutritious diets to maintain the high production merits and they need at least 38 dietary nutrients in appropriate balanced rations. Mishra and Shukla (2014) observed that quails consume 30 to 35 g per day but feed should always be available to them.

In South Africa, currently, a standard ration for growing and breeding quails is not available; however, a commercial game-bird diet can be fed to quails (Randall & Bolla, 2008). For optimal performance, quails should be fed a diet containing approximately 250 g/kg crude protein, 12.6 MJ/kg of metabolisable energy and 10 g/kg calcium for the first six weeks (Randall & Bolla, 2008) as shown in Table 2.2. However, the game-bird diets are expensive and hard to find, as a consequence, farmers resort to using a chicken starter ration (180 – 220 g/kg CP), although the quails would grow slowly. The dietary requirements for maturing quails are the same although the calcium and phosphorus levels need to be increased, specifically after five weeks of age, ground limestone can be added to the diets or can be provided separately.

Table 2.2. Nutrient requirements of growing Japanese quails (g/kg, unless otherwise stated)

Nutrient	Grower quails
Protein	180 – 240
Linoleic acid	10.0
Vitamin A (IU)	1.65
Vitamin E (IU)	12.0
Vitamin K (mg)	1.0
Biotin (mg)	0.3
Choline (mg)	2.0
Niacin (mg)	40.0
Calcium	8.0
Phosphorus	3.0
Sodium	1.5
Chlorine	1.4
Iodine (mg)	0.3
Manganese (mg)	60.0
Selenium (mg)	0.2
Zinc (mg)	25.0
Arginine	12.5
Histidine	3.6
Isoleucine	9.8
Leucine	16.9
Lysine	13.0
Methionine	5.0

Adapted from NRC (1994)

In hot weather conditions where feed intake is low, the calcium and phosphorus can be increased to 35 g/kg in order to maintain egg production. Consumption of a diet with high amount of energy will reduce feed intake, suggesting that the nutrient density in the ration should be properly balanced to provide appropriate nutrient intake based on the nutritional requirements and actual feed intake (Klasing, 2009). Quail rations can preferably be fed as crumbles to minimize feed wastage, experienced when mash diets are offered.

2.7 Nutritional composition, utilisation and importance of canola and soybean

2.7.1 Canola (*Brassica napus*)

The name canola refers to edible oil that is produced from the seed of any of several varieties of the *Brassicaceae* family. It is the cultivar of *Brassica napus*, *Brassica rapa* and *Brassica juncea*. Worldwide, canola is one of the most common sources of vegetable oils and the second most important oilseed crop after soybean (Pan *et al.*, 2011). A year later, Khajali and Slominski (2012) reported that canola oil ranks second amongst industrialized products derived from oil plants on a world production scale. Canola oil is a rich source of mono-unsaturated oleic acids and also contains considerable amounts of linoleic and alpha-linoleic acids, which are known to be the precursors of omega-6 and omega-3 fatty acids (Antongiovanni *et al.*, 2009). The term canola is a contraction of *Can*ada and *ola*, meaning oil. Spragg (2013) stated that the quality of canola meal (CM) depends on the type of oil extraction process (expeller-pressed and solvent-extraction). The CM from the expeller-pressed has more residual oils than the solvent-extracted CM. According to the Canola Council of Canada (2015), in order for the plant to be named "canola" the co-products must contain less than 2% erucic acid and less than 30 µmol/g glucosinolates.

Canola meal is currently used in poultry production mostly for feeding broilers and laying hens because it is a greater source of vitamins such as biotin, choline, folic acid, niacin, riboflavin and thiamine. Wickramasuriya *et al.* (2015) argued that CM has a comparable amino acid profile with SBM, which is an advantage for poultry feeding. Although it is limiting in lysine, it has high levels of methionine and cysteine. Another advantage is that canola is a readily available legume suggesting that it can be used as an inexpensive alternative protein source to reduce feed cost. Although, the crude protein (CP) content of CM ranges from 36–39% (Wickramasuriya *et al.*, 2015), lower than that of SBM, which ranges from 44–49% (Newkrik, 2010), it should be noted that CM has a well-balanced amino acid profile suitable for quails. Canola meal also has low digestibility of the essential amino acids when compared to SBM, which could be due to enzyme inhibitors and other antinutritional factors (ANF) present in the canola. It is, therefore, important that quail diets be formulated on the basis of the digestible amino acids in order to improve growth performance.

Inclusion of CM up to 30% in birds' diet compromise the productive performance, external and internal quality of eggs and the health status of the birds (Naseem *et al.*, 2006; Mushtaq *et al.*, 2007; de Oliveira-Moraes *et al.*, 2015). This is because canola contains several ANF that interfere with digestion processes. One of the factors affecting nutrient bioavailability of canola is the fibrous hull of its seed, since quails have limited capacity to digest fibrous substrates. Canola meal also contains non-starch polysaccharides (NSP), commonly known to reduce nutrient availability and break down into toxic aglucons. According to Landero *et al.* (2012), CM has lower gross energy when compared to SBM, which can be attributed to the high non-digestible fibrous substrates and lignin that canola contains. When compared to SBM, CM contains more phosphorus although

approximately 65% of the phosphorus is in phytate form (González & Stein, 2012; Slominski *et al.*, 2012).

Nevertheless, with so much research on canola, most of the antinutritional components have been altered by genetic selection that has evidently decreased its undesirable components (Leeson *et al.*, 2001). According to the Canola Council of Canada (2009), CM can be used as a protein source for turkeys up to a level of 30%, which is suitable in the grower phase. A study conducted by Min *et al.* (2011) indicated that 25% level of CM can be used in broiler diets with no adverse effect on growth performance. Whereas, in a study conducted by Payvastagan *et al.* (2012), weight gain and feed conversion ratio were negatively affected by the inclusion of CM up to 20% in broilers. Similar research in Japanese quails is scanty thus the tolerance level of the quail to CM is unknown. Secondary plant compounds in canola such as polyphenolics, phytate, erucic acids and glucosinolates, limit the utilisation of CM as feed ingredient (Wickramasuriya *et al.*, 2015) in most avian species. The phytate in canola constrain the availability of phosphorus, which also interferes with the utilisation and digestibility of other minerals. Low levels of phosphorus can lead to poor development and growth of Japanese quails, suggesting that inclusion of phytase enzymes in canola-based diets may be a solution to the problem of phosphorus bioavailability.

2.7.2 Soybean (*Glycine max*)

Soybean is a widely consumed legume crop with a worldwide economic impact of $114 billion (Vagadia *et al.*, 2017). Soybean meal (SBM) is recognised as an exceptional source of supplemental protein in diets of pigs, dairy cows, poultry and even human beings (Peter *et al.*, 2001; Stein *et al.*, 2008). This legume crop is an outstanding protein source (44–49%), largely known as the gold standard because, generally, all plant protein sources are

often compared to it (NRC, 1994). The major soybean proteins are glycinin and ß-conglycinin. Cromwell (1999) reported that SBM contains highly digestible protein, which is composed of a superior blend of amino acids (AA) that are ideal for most avian species. Soyabean products are by far the most popular plant protein and AA sources in livestock diets, with other products such as full-fat soybeans, soy protein concentrate, soy protein isolate, soybean oil and soybean hulls used worldwide by different sectors, as indicated in Figure 2.1.

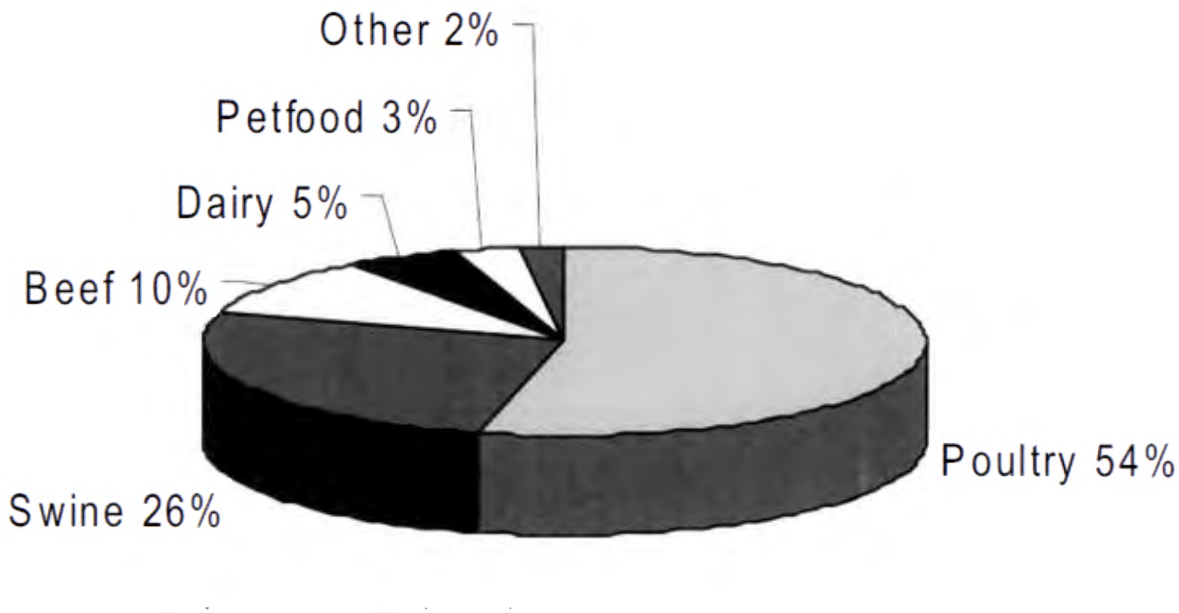

Figure 2.1. Worldwide use of SBM by livestock, poultry and companion animals (Source: Stein *et al.*, 2008)

When compared to canola, SBM is a rich source of lysine, tryptophan, threonine, isoleucine, and valine, although they are seriously deficient in poultry-based diets. The challenge of feeding SBM in poultry is that it tends to be low in methionine and cysteine, which makes canola a suitable replacement as it contains high levels of these AAs. Generally, lysine is used to compare the nutritional value of CM and SBM because it is the first limiting amino acid for pigs. However, in poultry, methionine is first limiting and lysine comes second. Usually, SBM has approximately 100 g/kg free sugars (Choct *et al.*,

2010), 60 g/kg soluble NSP, 180–210 g/kg insoluble NSP, and less than 10 g/kg starch (Knudsen, 1997). Raw soybean products contain significant amounts of ANF, especially trypsin inhibitors (TI) that are widely known to inhibit the digestibility of protein and reduce AA bioavailability in the gastro-intestinal tract. Several scholars have stated that heating SBM can reduce the activities of TI (Ravindran & Amerah, 2008).

Table 2.3. Chemical composition of canola versus soybean meal (g/kg, unless otherwise stated)

Component	Canola meal	Soybean meal
Crude protein	360 – 390	440 – 490
Erucic acids	< 20	N/A
Glucosinolates (μmol/g)	< 30	N/A
Metabolisable energy (kcal/kg)	2070	2230
Non-phytate P	3.8	2.8
NSP	180	178
Phosphorus (P)	10.2	6.6
Phytate P	6.4	3.8
Phytic acid	33	10 – 15
Sinapine	10	N/A
Starch	24	< 10
Total fibre	324	218
TI activity (TIU/mg)	17.7	< 14.0

Sources: NRC (1994); Canola Council of Canada (2009); Newkirk (2010); Khajali & Slominski (2012); Wickramasuriya *et al.* (2015); Hussain (2015)

Nevertheless, extra care must be provided to adequately heat the SBM as overheating will cause the AA, especially lysine, to bind with carbohydrates and form complexes and thus reduce their digestibility. In addition, AAs can be damaged by overheating (Cromwell, 1999). Soybean also contain non-starch polysaccharides (NSP), namely: arabinans, arabinogalactans and acidic polysaccharides. In poultry, NSPs are reported to disrupt the absorption of nutrients associated with increased viscosity of the intestinal content (Bedford & Classen, 1992; Almirall *et al.*, 1995), and also reduce blood cholesterol (Svihus *et al.*, 1997).

2.7.3 Amino acid composition and digestibility of canola meal versus soybean meal

Protein quality for simple non-ruminants is defined as the ability of a feedstuff to provide sufficient amount of digestible essential amino acids. Dietary protein requirements are a reflection of AA requirements used by animals to meet a range of body functions. For instance, AAs are primary components of structural and protective tissues (skin, feathers, bone matrix, and ligaments) and soft tissues (organs and muscles). It is for this reason that protein supplement constitutes the largest component during diet formulation (Beski *et al.*, 2015). Inclusion of SBM in quail diets is expensive due to its high market prices. Finding, therefore, alternative dietary protein sources that will replace SBM in poultry diets and reduce feed costs is of paramount importance.

Canola is considered as one of the potential alternatives because of its high CP content and well-balanced AA profile (Mariscal-Landin *et al.*, 2008), which is comparable to soybean. Aidera & Barbanab (2011) reported that the proteins have high arginine, leucine and glutamine contents but low quantities of sulfur-containing amino acids. Excessive heating during processing causes alterations on the AA profile of canola (González-Vega & Stein, 2012). For example, Adewole *et al.* (2016) reported a reduced concentration of lysine

during a heating process, which can be a result of Maillard reactions that occur between amino acids and reducing sugars when heat combines with moisture (Nursten, 2005). Newkirk *et al.* (2003) reported that the maximum temperature of 107°C normally used during desolvetization causes protein denaturation, whereas processing at a temperature of 100°C increases lysine digestibility to levels similar those reported for SBM.

The digestibility of AAs varies according to the nature of the dietary source, for example, a variety of canola with high protein content exposed to high temperature processing would have high digestibility of lysine and cysteine compared to those with low CP content. Amino acid digestion and absorption serve a variety of metabolic functions, which is the reason why growth and productivity stagnate when dietary AA is inadequate. Amino acids are normally classified into essential and non-essential (Nursten, 2005). The essential AA are those that the quail cannot synthesise at all or synthesise them at a slowest rate that is not enough to meet metabolic requirements, while the non-essential AA are those than can be synthesized from other amino acids (González-Vega & Stein, 2012). For this reason, supplying essential AA to high-performing animals should be prioritised and this can be achieved through the use of protein sources, whose AA composition is highly digestible. Furthermore, sufficient amounts of non-essential AA in the diet would reduce the necessity to synthesise them from essential AA (Mariscal-Landin *et al.*, 2008). The use of protease feed enzymes in improving AA digestibility is dependent on the protein ingredients used in feed formulation. Table 2.4 shows the differences in the total AA concentrations of soybean and canola meals.

Table 2.4. Comparison of total amino acid concentration (g/kg, as-fed) and digestibility coefficients (g/kg) between soybean and canola meal

Amino acids	Soybean meal		Canola meal	
Essential AA	Concentration	Digestibility	Concentration	Digestibility
Arginine	35.3	917	25.5	846
Histidine	12.6	894	10.3	820
Isoleucine	22.6	870	16.4	768
Leucine	37.8	875	28.7	786
Lysine	31.3	891	23.1	769
Methionine	6.8	904	7.2	819
Phenylalanine	24	885	1.74	802
Threonine	18.2	851	15.9	734
Tryptophan	7	880	5	820
Valine	2.4	858	2	757
Non-essential AA				
Alanine	20.4	873	17	781
Aspartate	55.2	863	33.7	768
Cysteine	7.7	830	8.7	770
Glutamine	85.6	903	66.6	853
Glycine	20	-	18.1	-
Proline	22.1	884	20.4	788
Serine	20.6	889	15.3	799
Tyrosine	17.8	889	11.6	775

Adapted from NRC (1994) and Kim *et al.* (2012)

According to Khosravi *et al.* (2016) SBM is deficient in methionine, and this implies that when used as a sole protein supplement in poultry, synthetic methionine should be added to meet the dietary needs of the birds. Conversely, when CM is used, no synthetic methionine is required because it has a high methionine content, however, it is necessary to add synthetic lysine and threonine to meet the birds' ideal amino acid balance while minimizing protein content of the diet.

2.8 Antinutritional factors in canola and soybean meal

Soybean meal and canola meal contain various anti-nutritional factors (ANF) that can compromise the health status and reduce the performance of quails if no precautions are taken before feeding. Historically, the use of CM has been restricted in poultry diets because of its high fibre content and the presence of ANF such as glucosinolates, erucic acids, polyphenolic substances (sinapine and phytate), non-starch polysaccharides, phytic acids and trypsin inhibitors (Canola Council of Canada, 2009; Hussain, 2015; Wickramasuriya *et al.*, 2015). The use of canola can also pose a threat to Japanese quails, if secondary plant compounds are not reduced. Overconsumption of these ANFs can reduce voluntary feed intake and growth rates, and alter pathophysiological status of the birds, usually pronounced by liver damage, abnormalities, and increased mortality rates (McNeill *et al.*, 2004). Canola also contains secondary compounds that limit the bioavailability and the utilisation of protein, carbohydrates, phosphorus, calcium and sodium and thereby cause imbalances between the synergy of these nutrients. Nutrient imbalances are reported to lead to poor gain: feed ratio, poor structural development and stagnated growth. Proper pre-treatments (cleaning, cracking and cooking) or processing of these protein sources during feed formulations is necessary to avoid consumption risks and toxicities (Newkirk, 2010).

2.8.1 Phenolics

Canola seeds have high concentrations of polyphenolic compounds when compared to other oilseeds (Naczk *et al.*, 1998). Polyhenolic compounds can be categorized into non-tannin and tannin phenolics and have influence in feed utilization and animal health status. The tannin group is classified, according to their chemical structures, into two fractions namely: hydrolysable tannins (tannic acid) and condensed tannins (proanthocyanidins) (Yapar & Clandinin, 1972). Hydrolysable tannins consist of gallic acid and its dimeric condensation product, hexahydroxydiphenolic acid esterified to a polyol, which is mainly glucose.

Bate-Smith and Ribereau-Gayon (1959) first discovered the presence of condensed tannins (CT) in rapeseed hulls, and Durkee (1971) verified their presence by identifying cyanidin, and pelargonidin in the hydrolytic products of rapeseed hulls. In addition, the presence of CT (leucocyanidin) was reported in rapeseed hulls by Leung *et al.* (1979). Canola meal contains 0.68 to 0.77% CT (Shahidi & Naczk, 1988; Naczk *et al.*, 2000). Naczk and Shahidi (2004), identified sinapine (flavones, isoflavones and anthocynidin) as the major phenolic compound of rapeseed, accounting for 1 to 2% (w/w) of the whole rapeseed (Khattab *et al.*, 2010) as shown in Figure 2.2. Lee *et al.* (2008) identified the major polyphenolic compounds in soybean flour namely: ferulic, syringic, and vanillic acids.

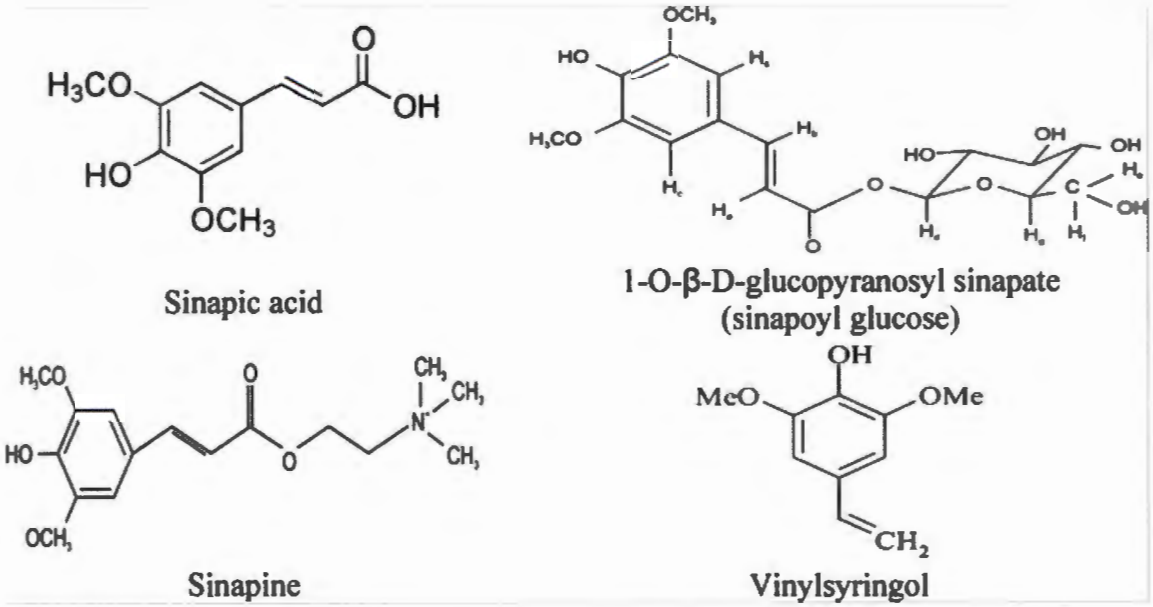

Figure 2.2. Chemical structures of the key phenolics present in canola (Source: Naczk *et al.*, 1998)

Alu'datt *et al.* (2014) reported that polyphenolic compounds have deleterious effects that are formed by their chemical nature, which allows them to conjugate with other nutrients such as vitamins, minerals, proteins, lipids and carbohydrates. Canola seeds have higher amounts of phenolic compounds, particularly CT, compared to soybean seeds, whereby a great proportion of CT are water-insoluble and located in the cells of hull fraction (Jia *et al.*, 2012; Khajali & Slominski, 2012). Canola hulls have a lot of insoluble tannins, ranging from 70 to 96% of the total tannins.

Leslie *et al.* (1976) reported that the inclusion of tannic acid (1.5%) severely depresses growth rates in broilers. However, Khajali and Slominski (2012) demonstrated that the antinutritive effect of tannins in canola should be minimal because tannins in canola are mostly water-insoluble and are located within the hull fraction. According to Xiao *et al.* (2011) phenolics and flavonoids play a pivotal role with regards to their health benefits to animals as they have anti-inflammatory, antioxidant and anticancer properties. The decarboxylation of sinapic acids in canola through the roasting processes results in the

production of canolol (Spielmeyer *et al.*, 2009; Siger *et al.*, 2013), which is a phenolic compound found in crude canola oil (Wijesundera *et al.*, 2008) as shown in Figure 2.3.

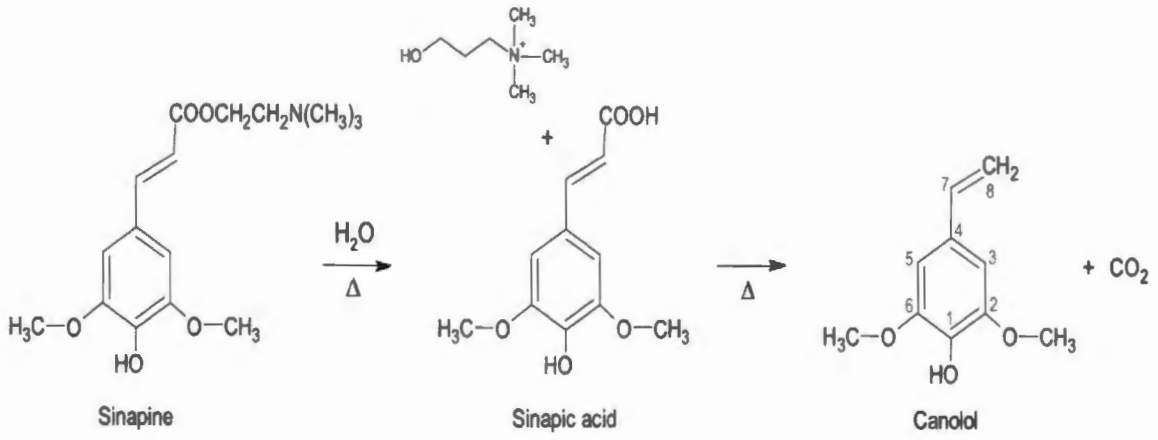

Figure 2.3. Chemical structures of sinapine transformed to sinapic acid and its decarboxylation to Canolol (Source: Li & Guo, 2016)

2.8.2 Erucic acids

According to Nath *et al.* (2009), erucic acid can be described as a long chain polyunsaturated fatty acid consisting of 22 carbon atoms with a double bound at the *cis*-13 position of the carbon chain (*cis*-13-docosenoic acid, C22:1) as indicated in Figure 2.4. Erucic acids are some of the main fatty acids found in canola oil and they are considered as some of the undesirable secondary plant compounds restricting the use of canola in poultry nutrition (Chen *et al.*, 2015). Rapeseed cultivars containing high levels of erucic acid have gained the attention of industrial sectors because erucic acids and their derivatives are important renewable raw materials for the production of plastics, lubricants, soaps, printing inks, and surfactants (Carroll, 1953; Aidera & Barbanab, 2011). However, in the agricultural industry, particularly poultry farming, consumption of diets containing high levels of erucic acids causes myocardial lipidosis and cardiac steatosis or heart lesion (Charlton *et al.*, 1975). It is for this reason that the Canadian researchers genetically modified the rapeseed plants to develop a low erucic acid variety in 1968.

Following these modifications, another double low variety was further developed in 1974, which is largely called "double low" or "double zero" interchangeably, referring to the low levels of erucic acids (< 2%) and glucosinolates (< 30 μmol/g). In 1979 (five years later), these low varieties were named 'canola' representing varieties containing less than 2% erucic acid and less than 30 μmoles glucosinolates. However, new reports have highlighted possible toxicities as a result of excessive ingestion of erucic acids despite the development of varieties with low levels of erucic acid (Sczaniecka *et al.*, 2012; Imamura *et al.*, 2013), suggesting the need to further develop varieties with even lower levels of erucic acids to avoid potential public health risks and to monitor the health status of birds being fed canola.

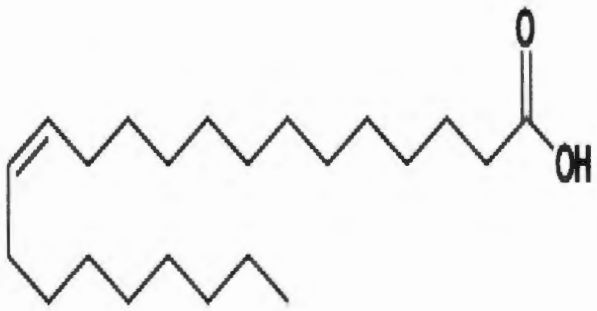

Figure 2.4. Chemical structure of erucic acids (Source: wildflowerfinder.org.uk)

2.8.3 Phytic acid

According to Jain and Singh (2016), phytic acids have acquired the name myo-inositol because they are not only the primary storage form of phosphorus, but also inositol in the bulk of grains (Figure 2.5). The exact role of phytic acids in animal nutrition is not clear (Khajali & Slominski, 2012). However, they are being considered one of the canola's antinutritional factors because they bind with proteins and several minerals (Ca, Fe, Zn, Mn and Mg) to form insoluble complexes, prompt nutrient excretion, and thereby reduce nutrient bioavailability and digestibility (Cabahug *et al.*, 1999; Bedford, 2000; Cowieson

et al., 2004). As stated by McCurdy and March (1992), the phytic acid content of CM ranges from 3.1% to 3.7%. Phytic acid reduces bioavailability of dietary mineral elements (Ford *et al.*, 1978) and inhibits several enzymes (e.g. α-amylase (Sharma *et al.*, 1978), trypsin, tyrosinase and pepsin (Graf, 1986)) and subsequently reduces animal performance. In canola more than 60% of the phosphorus is organically bound to phytate and phytic acid, which is a challenge for Japanese quails because they lack the necessary enzyme, phytase, in their digestive tract to break down phytate and release the phosphorus for absorption (Jain & Singh, 2016). As a result, the ingested phytic acid results in excess amounts of phosphorus being excreted into the environment causing eutrophication (Mullaney & Ullah, 2003; Vats & Banerjee, 2004; Singh & Satyanarayana, 2015). Not only does phytic acid increase phosphorus excretion, it also causes increased sodium excretion in poultry birds (Cowieson *et al.*, 2004). Besides limiting phosphorus bioavailability, phytates pose ecological problems as the excreted phosphorus pollutes the environment (Liu *et al.*, 2016).

In plant seeds, phytate acts as a storage form of phosphorus (Selle *et al.*, 2000), and is known to interact with several minerals to form phytate-mineral complexes (Cervantes *et al.*, 2011). Poor utilisation of phosphorus by quails can cause imbalances between calcium and phosphorus, and subsequently lead to poor structural development. According to Khajali and Slominski (2012), the phytic acid content of canola is (36–70%) can be broken down by the use of phytase, an enzyme that hydrolyses phytic acid to inositol and inorganic phosphorus. The use of commercially produced phytase enzymes has improved phosphorus bioavailability and utilization and increase overall growth performance in quails.

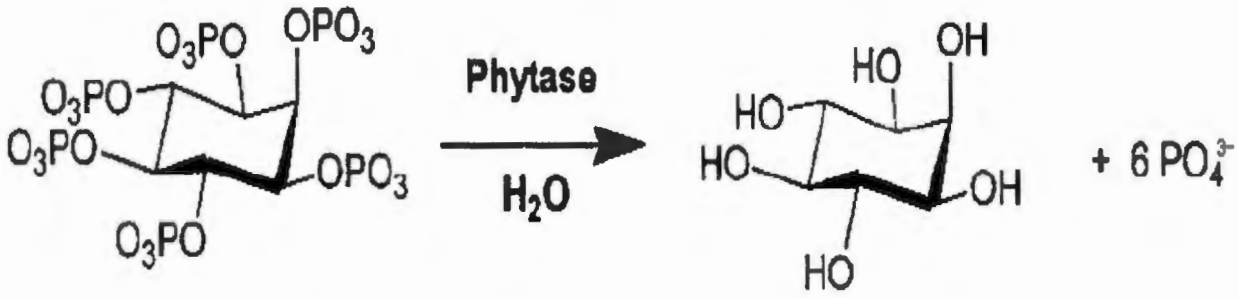

Figure 2.5. Hydrolysis of phytic acid by phytase to release inositol (Adapted from Jain & Singh, 2016)

2.8.4 Glucosinolates

Glucosinolates are the major ANF present in canola, and they are classified into two major groups, namely: aliphatic and aromatic glucosinolates (Tripathi & Mishra, 2007). *Brassica juncea* canola co-products have larger content of aliphatic glucosinolates compared to *Brassica napus* canola co-products. Degradation products of aliphatic glucosinolates are poisonous, whereas for the aromatic glucosinolates toxicity has not been clearly established (Tripathi & Mishra, 2007).

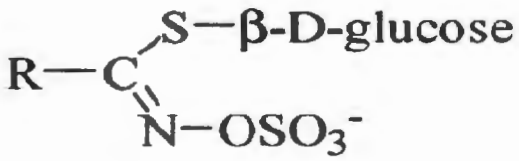

Figure 2.6. Chemical structure of glucosinolates (Source: Tripathi & Mishra, 2007)

Naturally, glucosinolates occur in plant tissues in close proximity to the enzyme myrosinase (thioglucoside glucohydrolase, EC no. 3.2.3.1), which, in the presence of moisture, hydrolyses glucosinolates into various toxic products such as aglucones, isothiocyanates, nitriles and thiocyanates (Mithen, 2001; Tripathi & Mishra, 2007). These toxic products are known to disturb the function of the thyroid gland and negatively affect

growth performance (McCurdy, 1990). Glucosinolates can be toxic and have to be detoxified by the liver and kidney, therefore, their consumption increases metabolic activities in the liver and kidney, causing hyperplasia, hypertrophy and necrosis of cells in these organs (De Groot *et al.*, 1991). Ingestion of glucosinolates can lead to liver damage, increased expenditure of energy by the liver, reduced feed intake and growth rate, and increased mortality (McNeill *et al.*, 2004). Although the concentrations of glucosinolates have already been reduced to low levels in CM, there is evidence that the conversion from rapeseed meal to CM has not yet totally eliminated liver toxicity (Campbell & Slominski, 1991).

Glucosinolates are the major ANF found in canola co-products, and poultry animals can tolerate up to 2.0 mol/g of glucosinolates in their diets thus dietary canola inclusion should be maintained below this level. Solvent-extracted co-products have lower glucosinolate content than expeller- or cold-pressed co-products due to the loss of some glucosinolates during desolventisation (Newkirk & Classen, 2002). Glucosinolates are bitter (Mailer *et al.*, 2008), which could be the reason for poor performance of birds (Slominski *et al.*, 1999; Jia *et al.*, 2012) with deleterious effects most pronounced in younger birds (Ahmad *et al.*, 2007).

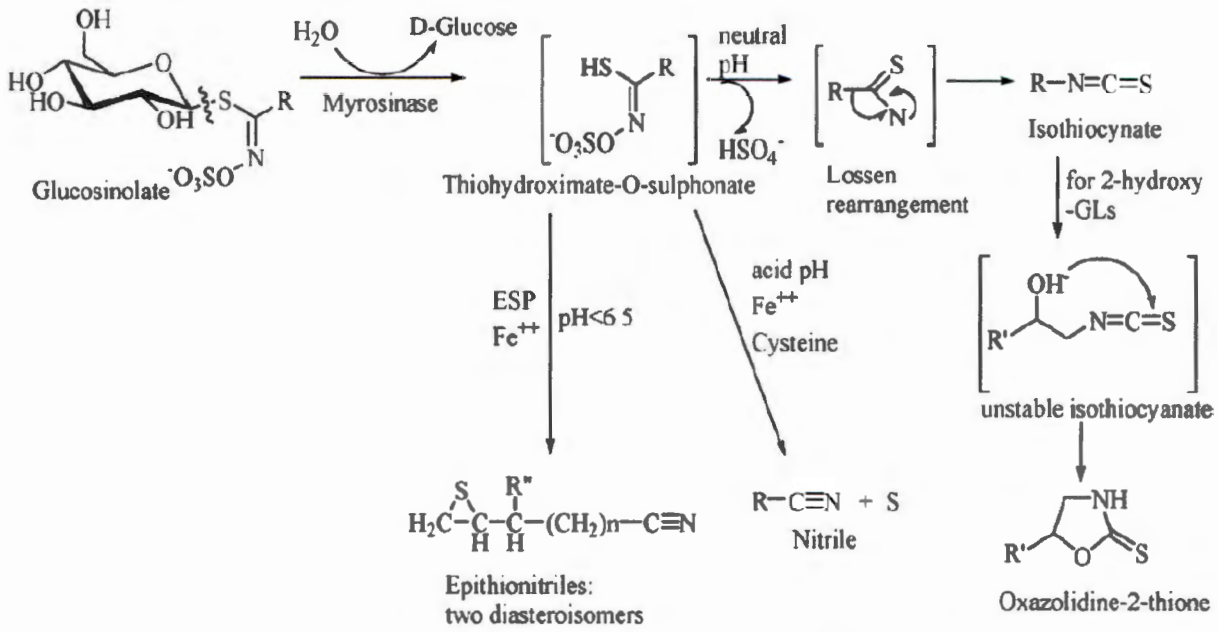

Figure 2.7. Enzymatic hydrolysis of glucosinolates by myrosinase (Source: Tripathi & Mishra, 2007)

2.8.5 Trypsin and trypsin inhibitors (TI)

Trypsin is a proteolytic enzyme that plays a pivotal role during the digestion of proteins in the quail. Trypsin is produced by the pancreas in its inactive form, trypsinogen, and gets activated during digestion as it enters the small intestine (Walsh *et al.*, 1964). Trypsin inhibitors (TI), also known as protease inhibitors (PI), are a group of serine protease enzymes that reduce the activity of trypsin and chymotrypsin enzymes, and may have antimicrobial, anticancer and anti-inflammatory activities (Kuhar *et al.*, 2014). They are classified into two major classes, namely: Kunitz trypsin inhibitors and chymotrypsin inhibitors (Pusztai *et al.*, 2004). The major trypsin inhibitors in soybean are Kunitz trypsin inhibitors, whereas chymotrypsin inhibitors are largely found in grain legumes (Jezierny *et al.*, 2010). Dietary TI bind to pancreatic digestive enzymes, trypsin and chymotrypsin, in the gastrointestinal tract to form inactive complexes, leading to reduced AA digestibility (Jezierny *et al.*, 2010), and thereby reduce growth rate and gain: feed ratio (Leiner, 1994). The secretion of trypsin in the pancreas is facilitated by cholecystokinin (CCK), hence TI

increases CCK production and as a consequence, reduce voluntary feed intake by reducing amino acids digestibility (Hara *et al.*, 2000; Ripken *et al.*, 2015). When diets containing TI are consumed, an irreversible trypsin enzyme-trypsin inhibitor complex is formed leading to a trypsin enzyme drop in the intestine, and thereby interfering in protein digestibility process (Cabrera-Orozco *et al.*, 2013).

The presence of TI does not directly affect the digestibility of the soybean consumed, it can also cause pancreatic hypertrophy or hyperplasia in the quail (Embaby, 2010). However, TI can be destroyed by heat during the processing of SBM (Ravindran, 2013a). Solvent-extracted SBM has low TI activity (< 14.0 TIU/mg) with most of the TI being destroyed during the desolventising-toasting stage of oil extraction. Poultry can tolerate TI levels up to 4.0 TIU/mg in the diet (Su & Chang, 2002), suggesting that precautions and accurate chemical analyses should be determined during feed formulations in order to avoid compromising the health status of the quails. Little is known about the presence of TI in canola because too much attention has been directed towards the reduction of glucosinolates and erucic acid. However, Berot *et al.* (2005) and Hussain (2015) have isolated and identified trypsin inhibitors in CM. Figure 2.8 illustrates the steps involved in the characterisation of trypsin/protease inhibitors from CM.

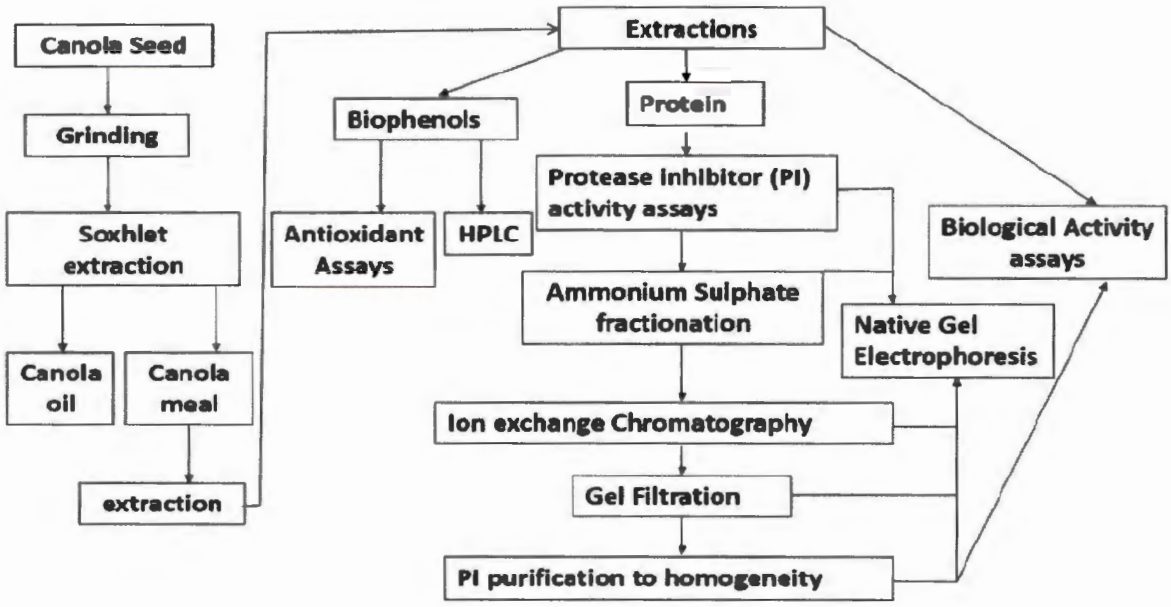

Figure 2.8. Protease inhibitors extraction steps from CM (Source: Hussain, 2015)

2.8.6 Non-starch polysaccharides

Non-starch polysaccharides (NSP) are a diverse group of indigestible carbohydrates composed primarily of plant cell wall components such as cellulose, pectins, β-glucans, pentosans, heteroxylans, and xyloglucan, which cannot be hydrolysed by endogenous enzymes of simple non-ruminants in general, and quails in particular (Sasaki & Kohyama, 2012; Kumar *et al.*, 2012). According to Woyengo (2016), NSP form the cell wall structure of various grains and legumes and they are referred to as dietary fibre that is resistant to degradation by mammalian enzymes. Indeed, Singh *et al.* (2017) reported that CM have indigestible substrates such as NSP that do not only decrease overall digestibility of feed, but also reduce utilization of other nutrients. According to Choct (1997) and Englyst (1989), they can be chemically analysed as crude fibre, neutral detergent fibre and acid detergent fibre.

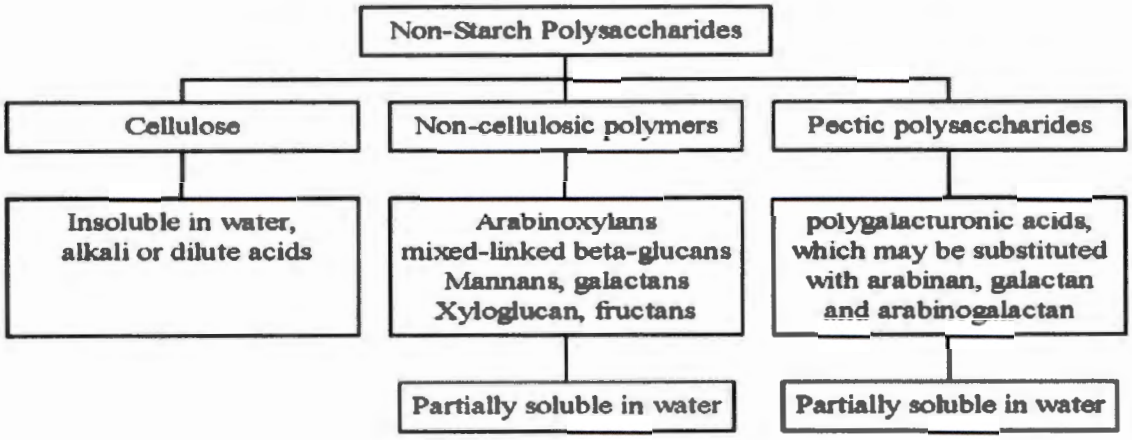

Figure 2.9. Classification of non-starch polysaccharides (Source: Choct *et al.*, 2010)

Non-starch polysaccharides differ from starch according to the number and type of monomeric units linked together, the order in the chain and the types of linkages between the various monomers. For example, starch is composed solely of glucose monomers linked together by α-glycosidic bonds, while NSP are composed of various kinds of monomers linked mostly by β-glycosidic bonds (Kumar *et al.*, 2012). The NSPs can be categorised into two groups, namely: soluble NSP and insoluble NSP. The soluble group of NSP is reported to increase the viscosity of digesta and reduce absorption of nutrients, while the insoluble NSP lowers digesta viscosity and result in faecal-bulking capacity (Davidson & McDonald, 1998), such attributes lead to anti-nutritive effects and poor performance in the quail. Classen *et al.* (1985) highlighted that high levels of NSP cause serious digestive problems in poultry.

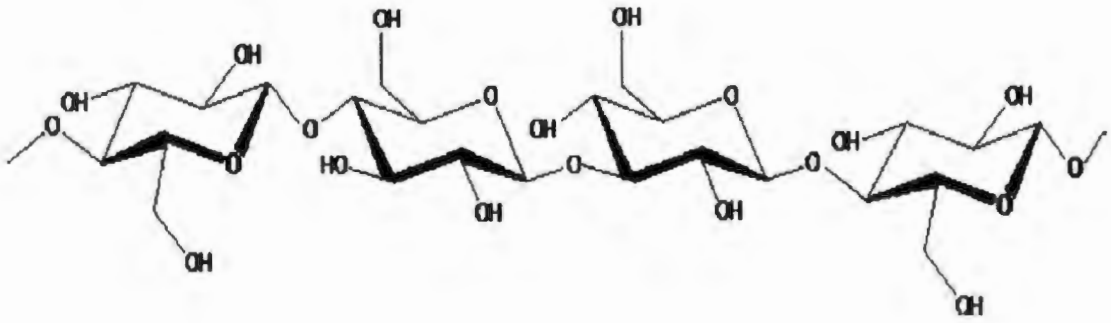

Figure 2.10. Chemical structure of β-D-glucans (Source: Ebringerova, 2006)

The presence of NSPs in poultry diets inhibits feed utilisation and decreases nutrient bioavailability, which adversely affect animal performance and slow down growth rate (Singh *et al.*, 2017). The use of NSP-degrading enzymes such as carbohydrase in feed is reported to mitigate the negative effects of NSP. For example, Slominski (2011) treated canola meal with carbohydrase enzymes and reported a reduction in substrate availability for harmful microbial growth in the ileum, and an improvement on nutrient digestion and absorption. Accurate blending of NSP-degrading enzymes produces low-molecular weight polysaccharides, simple sugars and oligosaccharides, which improve the gut environment by being utilized as prebiotics for beneficial microbes in the intestinal tract.

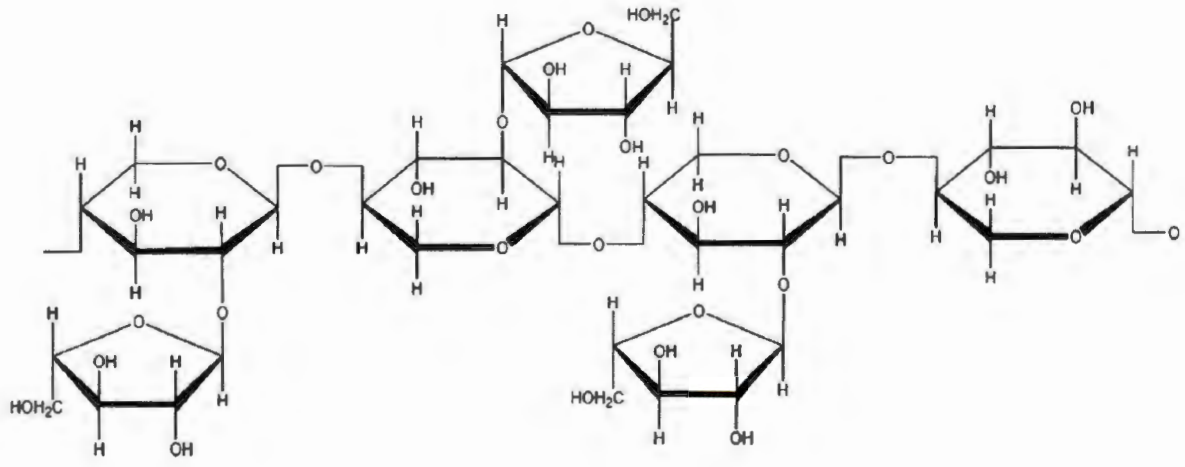

Figure 2.11. Chemical structure of arabinoxylan (Source: Ebringerova, 2006)

2.8.6.1 Glucans

Glucans are glucose polymers categorised according to their inter-chain linkage as alpha (α), beta (β) or mixed (α,β) patterns (Moreno-Mendieta *et al.*, 2017). Glucans play a crucial role in the biomedical and pharmaceutical industries due to their anticoagulant, antithrombotic, antioxidant, and anti-inflammatory properties (Kagimura *et al.*, 2015). Alpha-glucans are homogenous polysaccharides made of glucose monomers linked by α-glycosidic bonds (Moreno-Mendieta *et al.*, 2017). These α-glucans are largely present in all domains of life and perform significant roles as modulators of immune response. In α-glucans, glucose polymers (α-1, 4- and α-1, 6-linked branches) form the principal storage carbohydrates in plants cells. For this reason, α-glucans are usually not considered as bioactive molecules as β-glucans (Moreno-Mendietaa *et al.*, 2017). According to Zhu *et al.* (2016), β-glucans have been specifically accepted for their healing and immune-modulatory properties, as they form major bioactive compounds identified to possess biological activities such as anti-cancer, anti-inflammatory, and immune-modulating properties (Zhu *et al.*, 2016). Beta-glucans are a heterogeneous group of glucose polymers largely composed by the cell wall structural components of plants and some microorganisms such as fungi, bacteria and yeast (Moreno-Mendietaa *et al.*, 2017).

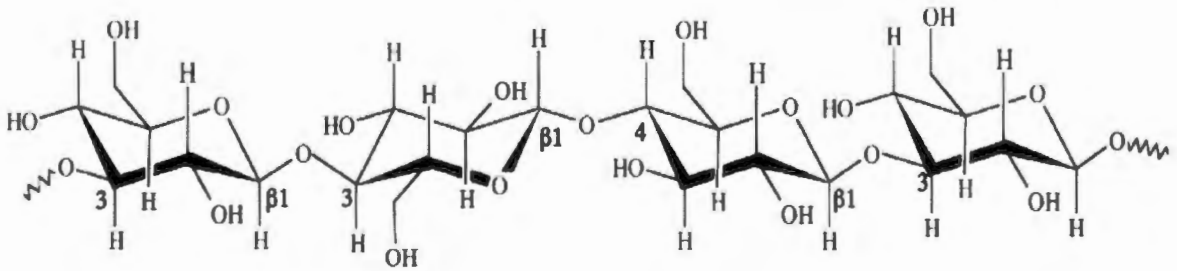

Figure 2.12. Chemical structure of β-glucan indicating the β-1, 4 and β-1, 3 linkages of glucose linked by glycosidic bonds (Source: Pillai *et al.*, 2005)

2.8.6.2 Xylans

Xylans are the major component of NSP in plant cells (Zijlstra & Beltranena, 2013; Woyengo *et al.*, 2014), indicating that their presence in canola elevates its fibre content and can reduce its utilisation. According to Kulkarni *et al.* (1999) as well as Shallom and Shoham (2003) xylan, cellulose, and lignin form the major polymeric constituents of plant cell walls, with the xylan being between cellulose and lignin. Due to their complex nature, their complete hydrolysis requires a large variety of xylans-degrading enzymes (Harris & Ramalingam, 2010). According to Bedford and Schulze (1998), hydrolyses of xylans is necessary to neutralize the anti-nutritive effects of dietary fibre. Therefore, application of exogenous enzyme xylanase is essential to cleave the backbone of xylan present in the canola and improve the quails' ability to utilise NSP-rich diets.

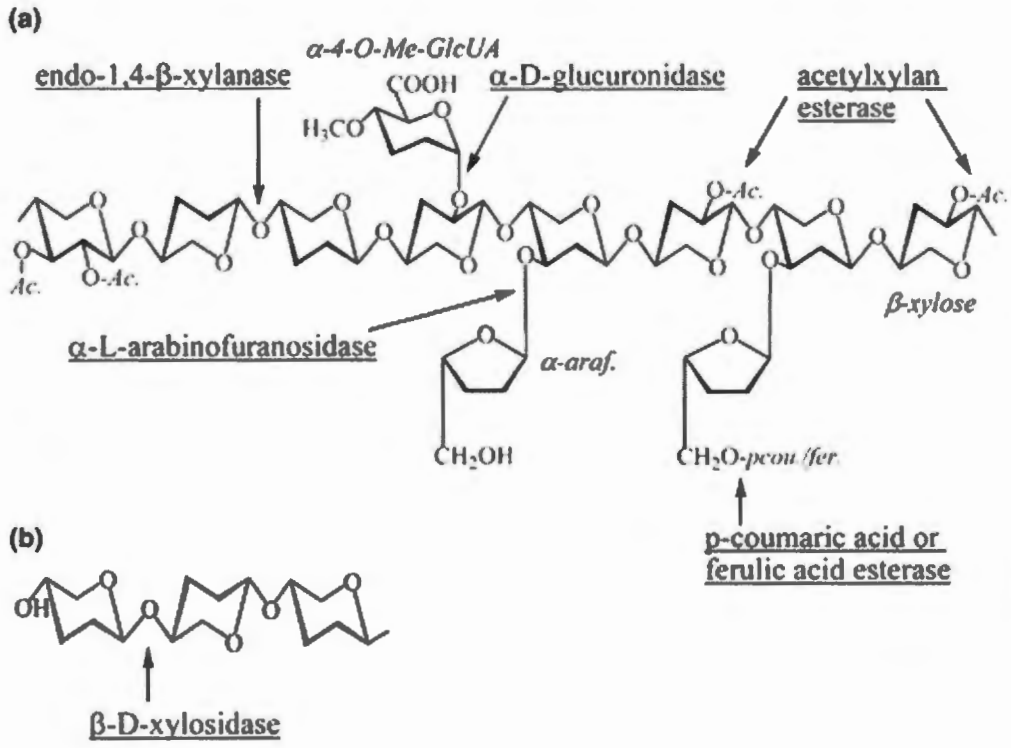

Figure 2.13. Chemical structure of xylan and how it is hydrolyzed by xylanolytic enzymes (Source: Sunna & Antranikian, 1997)

2.9 Application of exogenous enzymes

Recently, the use of feed additives, particularly enzymes, in animal nutrition has significantly increased as they play a very critical role in complementing endogenous digestive enzymes to enhance the digestibility of feed substrates, improve gut morphology, and increase growth performance of farm animals. This is because endogenous enzymes have a minimal or limited capacity to break down beta-linked carbohydrates and/or non-starch polysaccharides (Bedford & Cowieson, 2011). The limited capacity of mammalian enzymes to break down oligosaccharides, NSP and trypsin inhibitors in both canola and soybean meal suggests a need to evaluate the utility of exogenous enzymes such as carbohydrases and proteases in increasing nutrient bioavailability and digestibility of feed components. The application of exogenous enzymes in poultry diets positively alters gut health through changes to mucosal integrity, gut tensile strength, transportation of amino acids and environmental sustainability (Simbaya *et al.*, 1996).

According to Pariza and Johnson (2001), consideration of the appropriate production strain before using an enzyme is important to generate safe strain lineages, from which other strains can be derived through genetic manipulations. Ravindran (2013b) indicated that application of enzymes in young growing animals' diets during the first few weeks of life is essential as the digestive system is underdeveloped, therefore, young birds may benefit from a wide range of exogenous enzymes such as lipase, proteases, and carbohydrases. However, little is known about the use of exogenous enzymes in canola-based diets for Japanese quails. In order to close the gap, it is important to understand the target substrates that enzymes target in various feedstuffs. Table 2.5 shows, therefore, the different enzymes used in various feedstuffs.

Table 2.5. Enzymes and their target feedstuff and substrates

Target feedstuffs	Target substrates	Enzymes
Most plant-derived ingredients	Phytate	Phytases
Barley, oats and rye grass	β-Glucans	β-Glucanases
Wheat and other fibrous materials	Arabinoxylans	Xylanase
Soybean meal and grains	Oligosaccharides	α-Galactosidases
All plant protein sources	Proteins	Protease
Cereal grains and grains	Starch	Amylase
Lipids in feed ingredients	Lipids	Lipase
Fibrous plant materials	Cell wall matrix	Cellulase

Adapted from Ravindran (2013b)

2.9.1 Carbohydrases

Carbohydrases comprise all enzymes that catalyse a reduction in the molecular weight of a polymeric carbohydrates (Adeola & Cowieson, 2010). According to Ravindran (2013b), carbohydrases are enzymes that are widely used in poultry nutrition to cleave the viscous fibre components in NSP-rich feedstuffs such as the canola. Carbohydrases embrace numerous enzymes, however, xylanases and glucanases dominate the global carbohydrase market, with xylanases (endo-1-4-β-xylanase) carrying the enzyme commission (EC) identifier number: 3.2.1.8, and glucanases (endo-1–3(4)-β-glucanase) carrying the EC identifier number: 3.2.1.6. Both enzymes are in the same family, hydrolase, and subfamily, glycosidase (Adeola & Cowieson, 2011).

Generally, literature reveals little information on the supplementation of canola-based diets with enzymes in Japanese quails, particularly for the carbohydrases. Furthermore, there is a gap in knowledge about the types of carbohydrases and their enzyme activities used. For example, glucanase is usually termed as cellulase or glycosidase, which is partially correct because cellulase carry the EC identifier number: 3.2.1.4 and represents a series of glycosidase activities that depolymerize cellulose into glucose. On the other hand, xylanases are habitually called pentosanases, which are NSP-degrading enzymes, however, they are also not fully described. Carbohydrases such as α-amylase, β-mannanase, α-galactosidase and pectinase are commercially available but their use in animal nutrition is minimal (Adeola & Cowieson, 2011), this could be due to lack of information and also because of their high costs. Introducing exogenous carbohydrases into poultry diets is reported to improve the utilisation of dietary carbohydrates and also increase growth rates and NSP digestibility (Wang *et al.*, 2006). According to Graham and Pettersson (1992), the use of exogenous carbohydrases is necessary to reduce the nutrient encapsulating effect of the cell walls, and Chesson (1993) added that their use would further increase the nutritive value of feedstuffs by rendering the cell wall polysaccharides available for hindgut fermentation.

The effectiveness and activities of these enzymes can be influenced by several factors related to the enzyme source, biochemical characteristics, diet, animal and environmental conditions (pH, moisture and temperature). These factors singly or interactively influence responses observed with application of carbohydrases in animal feeding (Bedford & Schulze, 1998; Ravindran, 2013b). For example, Simbaya *et al.* (1996) reported that minimal activities of carbohydrase at pH 7.0 than at pH 5.3, this can be attributed to the fact that most fungal enzymes have ideal activity at a low pH and generally the optimum pH for most enzymes is between pH 4 to pH 6. Furthermore, Ravindran (2013b) stated

that for carbohydrases to perform accordingly, they should be exposed to aqueous or moist environments. This is because moist environments promote mobility of the enzymes and solubility of both the substrate and enzyme. Enzyme activities are generally known to increase to a temperature of 40°C followed by a sudden decline due to denaturing. Many enzymes denature with extreme temperatures and pH environments, rendering the enzyme inactive (Ravindran, 2013b).

Exogenous carbohydrases are known to modify the gut microbiota of birds by increasing the rate of digestion and limiting the amounts of substrates available to the microflora (Adeola & Cowieson, 2011). It is difficult to explain, therefore, the extent to which enzymes would exert their effects under the environment of the gastrointestinal tract because the resident duration of the digesta in compartments such as the crop, proventriculus and ventriculus is relatively short. Canola meal has high dietary fibre and about 18% of NSP present, therefore, treating the canola with a carbohydrase multi-enzyme would break down the cell wall structures of the NSP that cannot be hydrolysed by digestive endogenous enzymes, and reduce their antinutritive effects and thereby improve the quail performance. Application of carbohydrases in canola-based diets fed to Japanese quails is necessary to close the gap in literature.

2.9.2 Proteases

The use of protease feed enzymes was pioneered by Lewis *et al.*, (1955) and Baker *et al.*, (1956), who both reported improved weight gain in growing piglets. Over the past years, exogenous protease had formed part of commercial enzyme admixtures but it is currently available as mono-component enzyme (Cowieson & Adeola, 2005; Cowieson & Roos, 2016). The use of individual proteases as single enzymes has grown in profile although their effects on Japanese quails fed canola-based diets is scanty. The protease enzyme is

postulated to act in the plant cell wall by removing the structural proteins to allow faster digestibility.

Earlier studies indicated that the utility of proteases as supplemental feed enzymes was ambiguous; this is probably due to inconsistent findings and lack of information about the enzyme used. On many occasions, these inconsistencies in the efficacy of various exogenous proteases are difficult to interpret given the limited detail on the proteases used. For example, Simbaya *et al.* (1996) found considerable differences in efficacy of five alternative proteases in optimising broiler performance, but the enzymes were not fully described, in fact no enzyme activities were given. Furthermore, in an attempt to reduce the detrimental effect of proteinaceous antinutrients in soybean-fed piglets, Caine *et al.* (1997) first found that supplementary protease induced a decrease in AA digestibility. A year later, Caine *et al.* (1998) reported that supplemental proteases increase dietary protein digestibility by effecting the hydrolysis of certain proteins and thus enhance their solubility.

Blazek (2008) reported that the ability of protease enzymes to coagulate protein diets depends both on the type of dietary protein and the nature of the enzyme. According to Cowieson and Roos (2016), proteases liberate fat and starch, and also reduce the antinutritional effects of ANF present in the basal diet and thereby enhance intestinal integrity by emulsifying fats and improving endogenously produced mucin. In addition, feed protease is reported to improve the digestibility of AA (Romero *et al.*, 2013). However, there is no supporting evidence on the positive and/or negative alteration caused by exogenous proteases on endogenous secretions (Romero *et al.*, 2013). Marsman *et al.* (1996) reported that exogenous protease-treated soy-based diets had no effect on body weight gain and feed conversion ratio of chicks. Likewise, Yuan *et al.* (2017) reported no

effect on feed intake and weight gain when protease enzyme was added to broilers fed a corn-soybean basal diet. This is not surprising as Olukosi *et al.* (2007) also reported similar results. Nevertheless, other scholars have reached a common understanding on the ability of exogenous proteases to enhance nutrient bioavailability and thus improve weight gain and gain: feed ratio in simple non-ruminant diets (Mahagna *et al.*, 1995; Simbaya *et al.*, 1996; Ghazi *et al.*, 1997; Cowieson & Adeola, 2005; Cowieson & Ravindran, 2008). Differences on the observations and findings reported by different scholars can be attributed to different study designs and environments, various types of experimental animals and different dietary treatments. It is, therefore, important to determine the effect of the proteases in Japanese quails fed canola-based diets.

2.9.3 Phytase enzyme

Considerable amounts of phytic acid in the canola may hinder nutrient utilisation. Simons *et al.* (1990) reported that the inclusion of phytase in poultry diets is now a standard practice. Therefore, having a thorough understanding of the mechanism of action of the phytase and how it interacts with other enzymes is necessary for accurate interpretation of results. Phytase is a phosphatase enzyme that acts on monoesters (phosphomonoesterase) and is capable of hydrolysing phytic acid to produce inorganic orthophosphate and liberate myo-inositol (Irving & Cosgrove, 1972). Vohra (2003) and Singh *et al.* (2011) argues that phytases speed up the hydrolytic breakdown of phytate through a series of myo-inositol phosphate intermediates. Phytases are derived from many sources such as plants, animals and microorganisms (bacteria, yeasts and fungi), although the microbial-derived phytase the most reliable source (Ebune *et al.*, 1995; Rao *et al.*, 2009; Singh *et al.*, 2011). Like any other enzyme, there are several factors that impact on the effectiveness and utility of phytase such as the type and amount of phytase, method of pre-treatment and inclusion

(application), other feed ingredients and nutrient concentrations particularly protein and minerals.

Selle *et al.* (2015) reported that the understanding of the phytate-phytase axis is incomplete in poultry nutrition, precisely because the beneficial effect of exogenous feed phytases is due to the hydrolysis of phytate and the consequent enhancement of nutrient (minerals, amino acids, and energy) availability. Phytases increase the concentrations of AA in the gut lumen and thereby elevating relevant transport systems to increase the digestibility of protein. Truong *et al.* (2014) reported that phytase supplementation has resulted in sodium recovery along the small intestine of broilers. Phytases release inorganic phosphorus from phytic acid in plant-derived feed ingredients and eliminate the antinutritive effects and thereby increase phosphorus bioavailability (Selle and Ravindran, 2007).

Phytases improve the nutritional value of feeds and reduce phosphorus excretion, which is necessary for the reduction of eutrophication. Phytate-degrading enzymes enhance protein and energy utilisation (Selle & Ravindran, 2007; Oduguwa *et al.*, 2007), improves gut health reflected from reduced secretions from the gastrointestinal tract (Pirgozliev *et al.*, 2008), improve nutritional quality and reduce phosphorus contaminant levels in the environment (Jain & Singh, 2016). Ravindran (2013b) elucidated that the inclusion of phytase improves energy utilisation by increasing starch digestibility and by so doing energy availability increases, as starch is the main source of energy in poultry diets. Selle and Ravindran (2007) together with Xu *et al.* (2011) reported that phytase feed enzymes breakdown phytate-bound phosphorus by reducing and targeting the phytic acid complexes in plant ingredients and consequently increase the bioavailability of phosphorus and calcium.

2.10 Haematological parameters of Japanese quails

2.10.1 Indicators of bird health

Haematological parameters are essential indices used as indicators of pathological, physiological, health and nutritional status of animals (Ewuola *et al.*, 2004; Aro *et al.*, 2013), because they provide a clearer diagnosis and clinical monitoring of diseases in animals (Karesh *et al.*, 1997). Although haematological reference values there are numerous factors affecting these values. Haematological profiles vary according to the breed type, age, gender, stress, nutrition, season, bacterial and viral infections and poisoning (Khan & Zafar, 2005; Hall *et al.*, 2007).

Puspamitra *et al.* (2014) concluded that haematological parameters of Japanese quails rise with growth stage. In support, Addass *et al.* (2012) stated that the majority of haematological parameters (haemoglobin, red blood cell counts, white blood cell counts and haematocrit) for most birds increase with age and vary with gender, as males usually exhibit higher values than females. Furthermore, Esonu *et al.* (2001) added that haematological indices are important because they reveal the physiological changes of animals towards their internal and external environments, including the type of feed consumed and the behavioural feeding patterns.

It is important for haematological parameters to fall within the normal ranges or baseline reference data recommended for each species of animals in order to verify their harmonious well-being. This is because abnormal haematological values indicate poor health, illnesses and disease affecting an individual. Therefore, thorough knowledge on haematological indices and factors affecting them is crucial for accurate interpretation of results. For example, anaesthesia reduces haematocrit, haemoglobin and red blood cell

counts (Pilny, 2008), suggesting that when interpreting results it is important to consider the effects of anaesthesia on these haematological parameters.

According to Hussain *et al.* (2014) erythrocyte counts are the best biomarkers of oxidative stress, which reflect the physical and chemical alterations in different species. Njidda *et al.* (2006) reported that mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), widely referred as blood constants are used in the diagnosis of anaemic conditions. Normally, if MCV, MCH and MCHC values are lower than the recommended values that will be considered a sign of low blood levels hence important in diagnosing anaemia. Ahmed *et al.* (1994) observed that MCHC values decrease with increase in the level of protein.

According to Campbell and Lasley (1975), low lymphocyte counts indicate an inability or reduced ability of an animal to produce antibodies when infections occur. In contrast, higher values of lymphocytes and eosnophils than the reported standard values indicate that the animal's immune system was challenged by toxic substances or ANF present in the diets (Mitruka & Rawnsley, 1977). Furthermore, haemoglobin values falling within the normal range indicate a normal physiological relationship of haemoglobin with oxygen in the transport of gases across body tissues (Njidda *et al.*, 2006).

2.10.2 Dietary influence on haematological indicators

Improving the performance of animals, particularly quails, without compromising their health status, specifically haematological indices, is of economic importance. Poultry farmers tend to seek inexpensive alternatives to offer to their animals without taking precautions about the level of ANFs present or contained by the different feed ingredients that can negatively influence blood parameters. Studies on haematology provide a useful

tool in evaluating the effects of different nutrient rations on blood parameters of farm animals. Several scholars have reported the influence of different diets and impacts on haematological indicators of animals. Indeed, Swenson (1970) and Schalm *et al.* (1975) reported that nutrition affects haematological values of farm animals. Addass *et al.* (2012) posited that nutrition affects blood values of animals. Many researchers agree on diet impacting the blood profile of healthy animals (Iheukwumere & Herbert, 2002; Kurtoglu *et al.*, 2005; Aya *et al.*, 2013). Isaac *et al.* (2013) stated that the influence of nutrition on haematological components is pivotal for accurate assessment of feed toxicity, especially, with dietary constituents known to affect health status of animals.

Farm animals are subjected to changes in rations provided to them, but abrupt changes impose stress which in turn affects their metabolic, pathological and physiological statuses (Ewuola *et al.*, 2004). If animals are offered a diet containing high amounts of toxic substances, their health is compromised causing acute histopathological damage of body organs such as the liver, kidney and spleen (Ewuola *et al.*, 2003; Ewuola, 2009). Aletor and Egberongbe (1992) reported that erythrocytes and haematocrit are affected by diets, even though they mostly remain within the reported normal physiological ranges in most animals (Mitruka & Rawnsley, 1977). This is because haematocrit is correlated with the nutritional status of the animal. Therefore, studies conducted on haematological indices represent a beneficial and valuable tool in the diagnosis of many diseases, identification of disorders or conditions, as well as examination of damaged organs (Onyeyili *et al.*, 1991). The influence of canola inclusion based diets on haematological parameters of Japanese quail is scanty. Considering the reduction of glucosinolates and erucic acids in the canola as well as the presence of other ANF, it is not clear how the inclusion of CM would influence the haematological parameters of Japanese quails.

2.11 Serum biochemical parameters of Japanese quails

2.11.1 Significance of serum biochemistry in birds

Serum biochemical profiling is conducted to monitor the health of farm animals and diagnose subclinical disease (Ali *et al.*, 2012) because abnormal clinical conditions are associated with biochemical changes. For serum biochemical parameters to be most reliable as a useful diagnostic tool, there is a need to establish reliable reference values to detect changes in the health status (Verheyen *et al.*, 2007). According to Washington and van Hoosier (2012), serum is generally a preferred sample for clinical chemistry assays because during collection there are no anticoagulants required and thereby eliminate interference by the actions of an anticoagulant. For example, anticoagulants such as ethylene diamine tetra acetic acid and citrate inhibit coagulation by chelating calcium ions that are cofactors in enzyme assays. However, it is important to note that there are several factors that can alter the serum biochemical values before analyses are performed.

According to Mitruka and Rawnsley (1977) as well as Buetow *et al.* (1999), after collection the blood must be allowed to clot for 30 to 45 minutes at room temperature and thereafter refrigerated because parameters like glucose, phosphates, lipids, and chloride may change concentration at room temperature. However, serum storage conditions vary with analyses time, which means that samples stored at 4°C must be analysed within 24 to 48 h, those stored frozen at –20°C may be analysed within 90 days and those stored at –70°C may be analysed in 360 days or longer (Buetow *et al.*, 1999; Cray *et al.*, 2009). Although lactate dehydrogenase enzyme was reported to be unstable if the samples are frozen (Mitruka & Rawnsley, 1981). Saibaba *et al.* (1998) and Owens *et al.* (2005) reported that factors such as lipemia and hemolysis interfere with the results of serum biochemistry assays, producing high values for certain enzymes because most serum

enzymes (transaminases and lactate dehydrogenase) are present at high concentrations in red blood cells.

Muchenje *et al.* (2009) and Evans (2009) reported that diets, restraining and handling methods, drug administration, transportation and pre-slaughter stress causes biochemical changes in farm animals. It is, therefore, important to know the biochemical indicators associated with these changes. For example, increased levels of plasma amylase indicate tissue damage in salivary glands, pancreas and the liver because amylase is present in liver as well as salivary glands and also large amounts of alpha-amylase are produced from the pancreas (Evans, 2009). According to Washington and van Hoosier (2012), biochemical enzymes are usually contained within the cells that produce them, however a rise in enzyme levels in serum indicates cell damage. For this reason, elevated levels of aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are an indication of cellular necrosis, liver damage, hepatitis, congestive heart failure, and myopathy. This is because AST and ALT are contained within liver cells and they are used to measure liver function. Eugster *et al.* (1966) reported that, generally, AST levels increases after bruising, trauma, infection, or neoplasia of liver or muscle. While, the concentration of ALP is elevated by digestion, cholestasis, and injuries to intestinal or biliary epithelium, but these vary according to the type of species. Conversely, Fernandez and Kidney (2007) reported that levels of ALP decrease during fasting, hypothyroidism, or pernicious anaemia. Biochemical parameters such as total protein and its constituent albumin and globulin are very important as they are indicators of the body's defence mechanism.

According to Evans (2009), liver function can be habitually assessed using total serum protein and its constituents because serum proteins are sourced from the liver. Blood urea nitrogen, creatinine and uric acids are biomarkers of renal functions (Donsbough *et al.*,

2010). However, creatinine is a reliable indicator to measure renal function when compared to blood urea nitrogen because it is minimally affected by external factors such as diet and hydration. In addition, creatinine levels increase as a result of necrosis or atrophy of skeletal muscle, hyperthyroidism, infections, burns, or fractures (Mitruka & Rawnsley, 1981). Increased concentrations of blood urea nitrogen can be a result of feeding a high-protein diet or vigorous exercises, while decreased concentrations can be attributed to low protein intake and liver failure (McLaughlin & Fish, 1994). High concentrations of bilirubin indicate liver dysfunction or hemolytic disease, this is because bilirubin also serves as a measure of liver and bile tract function (Evans, 2009).

2.11.2 Dietary influence on serum biochemistry

Serum biochemical parameters serve as a practical diagnostic tool for evaluating and monitoring the pathological and physiological conditions as well as the health status of farm animals. As already explained, accurate diagnosis requires reliable reference values for the animal under diagnosis. However, literature reveals very little about the entire serum biochemical profile of quails. Indeed, Ali *et al.* (2012) stated that serum biochemical profiling has been limited by a lack of reference ranges, with most of the reported parameters based on small sample numbers. A further limitation is the fact that so many reports would focus only on individual specific parameters being affected by different factors such as nutrition, age, breed type, management and stress level (Ali *et al.* 2012).

According to Verheyen *et al.* (2017), comparing biochemical values reported in the literature is a challenge due to differences in study designs, type or breed of animals and analytical techniques, with some of the analytical techniques being outdated (Ali *et al.*, 2012). In essence, most of the reported biochemical values do not cover the whole serum

biochemical profile of quails. However, the effect of nutrition on serum biochemical parameters has been demonstrated on different farm animals. For example, Iyayi and Tewe (1998) observed that rabbits fed fumonisin-based diets had lower levels of serum proteins, suggesting that the diet have influenced protein metabolism, since the synthesis of serum protein depends on the amount of protein available in the diet. They also observed serum enzyme activities above the normal ranges, suggesting that the rabbits might have suffered liver and/or kidney damage, which was in line with the findings of Harper *et al.* (1997) as well as Ewuola and Egbunike (2008) who reported that increased concentrations of alanine aminotransferase and aspartate aminotransferase are clinical biomarkers of diagnosing damaged visceral organs. In a study conducted by Odetola *et al.* (2012), rabbits fed whole kenaf seed meal were reported to have anaemic conditions, damaged visceral organs and overall poor performance, which is a clear indication that ANF present in a diet alters the serum biochemical profile of animals.

Nazifi and Asasi (2001) reported that Japanese quails fed furazolidone were reported to have increased concentrations of cholesterol, uric acid, AST and ALP, whereas the concentration of total protein and calcium was reduced. Mohamed and Wakwak (2014), reported that supplementing quails with 4% of sesame seeds caused a reduction in the concentrations of some serum biochemical parameters such as total lipids, triglycerides and cholesterol. Mehri *et al.* (2015) found that Japanese quails fed dietary peppermint had reduced concentrations of triglycerides, total cholesterol and ALT, which indicated that adding peppermint negatively influenced the quails' blood profile. Mnisi *et al.* (2017) reported that Japanese quails supplemented with a *Lippia javanica*-based diet had increased concentrations of ALT. All these reported findings are a testimony that indeed nutrition has influence on serum biochemical parameters of quails and other research animals. For example, serum calcium and phosphorus are influenced by the type of diets

offered to the animals, it is therefore, likely that the phytic acid present in the canola would reduce the concentration of calcium and phosphorus in Japanese quails.

2.12 Effect of energy and protein on meat quality

Providing quails with sufficient amounts of dietary protein and energy is essential for their optimal performance. In turn, protein and energy availability influences the quality of the meat. Nutrient balancing prevents metabolic disorders and other undesirable attributes that negatively affect the quality of the meat. For example, altered rate of glycolysis and a low pH within muscle fibers results in abnormal muscle metabolism after slaughter causing pale soft exudative meat (Muchenje *et al.*, 2008). Pale soft exudative meat is characterised by an abnormal colour and poor water holding capacity, which eventually result in the meat appearing dry and unattractive. Excess amount of dietary energy is stored in the body as fat, which evokes public concern about the safety and quality of the meat (Webb & O'Neill, 2008). Woodgate and van der Veen (2014) demonstrated that consumption of fats from animal derived products increases the amount of saturated fats and the presence of cholesterol compared to plant-derived products, which is the reason why fat is considered unhealthy in many countries. Nonetheless, fats and fatty acids contribute greatly to various facets of meat quality.

According to O'Neil *et al.* (2012), the leading sources of dietary energy for animals are fats and carbohydrates, nevertheless proteins are still capable of providing dietary energy. Protein availability is reported to increase water-holding capacity because proteins have the capacity to retain more water (Muchenje *et al.*, 2009). In a study conducted by Li *et al.* (2015), who investigated how different dietary sources of energy affect muscle glycogen storage at slaughter, diets containing low amounts of starch and high amounts of dietary fibre reduced muscle glycolysis thereby improving meat quality, this is because meat

products have low carbohydrates and do not contain dietary fibre. These findings give an indication that inclusion of canola in the diets of Japanese quails would improve meat quality parameters because canola also has low starch and high fibre content.

Many studies have revealed that different sources of dietary energy have a significant impact on muscle glycogen storage at slaughter (Rosenvold *et al.*, 2001; Bee *et al.*, 2006), which affect the quality of the meat through the rate and extent at which post mortem pH drops. However, according to Joo *et al.* (2013), external feed manipulation has minimal effect on meat quality because the quality of meat is largely dependent on internal myofibre types. Teye *et al.* (2006) showed that isoenergetic diets of different protein content of 18% and 20% CP increased the concentration of total lipids. Indeed, Wood *et al.* (2008) reported that low protein diets limit muscle deposition, because the energy that would have been used for muscle synthesis is diverted to fat synthesis. When compared to soybean, canola has low AA digestibility, suggesting that inclusion of CM in quail diets might negatively influence protein quality of the meat. This further suggests the need to use exogenous proteolytic enzymes to increase the digestibility of AA and improve the texture of the meat.

2.13 Summary

Quails are the smallest avian species noted for fast growth rates, early sexual maturity and market age, short generation intervals, profilic laying and resistance to several avian diseases (Kabir, 2013; Dauda *et al.*, 2014). Their meat produce is now dominating luxury markets due to its tender taste (Randall & Bolla, 2008). In order to profitably improve quail production to meet anticipated growth in demand, there is a need to find alternative protein sources that can be used to substitute soybean, which is widely used as an excellent source of protein and digestible amino acids. Finding alternative protein sources

that are not used directly by humans and can only be consumed by domestic animals is a major priority for researchers.

Canola meal, a by-product after oil extraction has been reported to be a potential protein source (36–39% CP) and is noted for its AA profile that is comparable to that of soybean. However, canola contains antinutritional factors such as glucosinolates, erucic acid, non-starch polysaccharides (NSP), phytic acid, trypsin/protease inhibitors and polyphenolics that are known to interfere with digestion and negatively affect growth performance (Newkirk, 2010; Khajali & Slominski, 2012; Hussain, 2015). These factors affect the utilisation of canola in poultry diets with several scholars reporting that canola is a potential protein source but inclusion levels greater than 250 g/kg can cause detrimental effects and reduce performance in the birds (Ahmad *et al.*, 2007; Min *et al.*, 2011). If canola is to be included at higher levels, there is a need to find alternative strategies that can be employed to improve its feed value and reduce its antinutritive effects. Although, genetic modifications have reduced the levels of glucosinolates (< 30 μmol/g) and erucic acids (< 2%) (Canola Council of Canada, 2015), there is a need to further eliminate the effects of the other ANF.

The use of feed additives, particularly exogenous enzymes, in animal nutrition has been adopted to complement endogenous digestive enzymes. The inclusion of feed enzymes enhances the digestibility of feed substrates, improve gut morphology, and increase growth performance of farm animals. In order to reduce the effects of NSP and protease inhibitors present in the canola, the use of enzymes such carbohydrases and proteases is essential. Exogenous carbohydrases are known to cleave the viscous fibre components in carbohydrate-rich feedstuffs such as xylans and glucans and thereby increasing their

digestibility (Adeola & Cowieson, 2011). It is for this reason that the global carbohydrase market is dominated by xylanases and glucanases enzymes.

Protease enzymes have been used as part of enzymes admixtures for the past decades, but it is now widely available as a mono-component enzyme (Cowieson & Adeola, 2005; Cowieson & Ravindran, 2008). Protease feed enzymes are known to reduce the activities of trypsin/protease inhibitors that interfere with the function of pepsin and trypsin (Berot *et al.*, 2005) and thereby reduce protein and amino acid digestibility. The incorporation of feed enzymes in poultry diets increases energy utilization, nutrient digestibility and thereby enhances growth performance (Romero *et al.*, 2013; Stefanello *et al.*, 2016).

Literature also reveals incidences where inclusion of feed enzymes did not improve feed utilisation and animals' performance (Meng & Slominski, 2005; Mushtaq *et al.*, 2007). Information on supplementing canola-based diets with feed enzymes, particularly carbohydrase multi-enzyme and protease mono-component enzyme to enhance feed utilisation, growth performance, haematological and serum biochemical parameters, carcass and meat quality characteristics of Japanese quails is scanty. It is, therefore, important to investigate the effects of feed enzymes on the utilisation of canola-based diets in quails.

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3 CHAPTER THREE – CANOLA MEAL AS A REPLACEMENT FOR SOYBEAN MEAL IN JAPANESE QUAIL DIETS: GROWTH PERFORMANCE, HAEMO-BIOCHEMISTRY, CARCASS AND MEAT QUALITY TRAITS

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Abstract

This study was conducted to investigate the effect of partial replacement of the major dietary protein source, soybean meal, with canola meal on growth performance, haematology, serum biochemistry, and meat quality characteristics of female Japanese quails over a 4-week feeding period. A total of 140, 5-week old quails (158.28 ± 11.919 g live-weight) were randomly allocated to five isonitrogenous and isoenergetic experimental diets formulated by replacing the soybean meal component with canola meal as follows: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal. Experimental diets and fresh water were offered *ad libitum*. Average weekly feed intake (AWFI) and body weights were recorded and used to calculate average weight gain (AWG) and weekly gain: feed ratio (GFR). At the end of the 4-week feeding trial, all quails were slaughtered in a local abattoir in order to assess carcass and meat quality characteristics. Blood was collected at slaughter for analysis of haematological and serum biochemical parameters. Repeated measures analysis showed no significant week × diet interaction effect on AWFI, AWG and GFR. Quails offered CM175 had the lowest (P <0.05) feed intake while no differences were

observed among the other four diets. However, there were no dietary effects on AWG, GFR, and haematological parameters of quails. All serum biochemical parameters were not influenced by experimental diets, with the exception of alkaline phosphatase. Carcass characteristics and dressing percentage of quails across diets were also found to be similar. There were significant dietary effects on length of small intestines. Meat from quails offered CM25 had the least cooking losses (16.63%) while meat from those offered CM125 had the highest losses (21.07%). Dietary treatments had no effect on peak positive force of quail meat. Similarly, no dietary effect was observed in meat quality parameters immediately post slaughter. However, quails on CM25 had the highest chroma (7.39) while those on CM125 had the lowest (3.58), 24 h post slaughter. It was concluded that canola meal can replace soybean in quail diets up to 125 g/kg without compromising the birds' growth performance, health and quality of meat. Inclusion levels beyond 125 g/kg promoted poor voluntary feed intake and thus may require the use of feed additives to enhance utilization by the quails.

3.1 Introduction

The poultry industry is one of the largest South African agricultural sectors, which evolved over the past 100 years from backyard household farming to highly sophisticated production units (Bolton, 2015). The industry continues to evolve with the addition of new bird species, such as the Japanese quail (*Coturnix coturnix japonica*), to complement the existing species. Quail farming, although a relatively recent addition to the South African poultry industry, already contributes high-quality dietary protein for human consumption (Khosravi *et al.*, 2016). Quail farming is economically viable and technically feasible because quails reach sexual maturity at 6 weeks of age, quite resistant to various avian diseases and easily adapt to various rearing conditions (Randall & Bolla, 2008). However, the major challenge in the long-term sustainability of the poultry industry remains the cost

of dietary protein and supply of essential amino acids (Wickramasuriya *et al.*, 2015; Rezaeipour *et al.*, 2016). Due to the nature of the avian digestive system (monogastrics), birds require dietary protein of very high quality, much similar to what humans require (Beski *et al.*, 2015). Therefore, there is direct competition between poultry and humans for soybean. This often results in relatively higher prices of soybean on the world market creating artificial food shortages among the poorest societies around the world. According to Scanes *et al.* (2004), the efficacy of a protein feedstuff for poultry depends on its capacity to supply adequate amount of essential amino acids as well as digestible protein required by the bird. The quality of soybean meal as a potential protein source for poultry is unquestionable. However, alternative dietary protein sources are required to help alleviate the challenge of high feed costs encountered in poultry production. Canola meal is one such relatively inexpensive protein source (34–39% CP) that has potential to be used as an alternative to soybean (40–48% CP) in poultry diets (Mushtaq *et al.*, 2007; Beski *et al.*, 2015). However, canola contains antinutritional factors such as trypsin inhibitors, non-starch polysaccharides and phenolics, and also has high fibre content (Bell, 1993; Swick, 1999). These components reduce amino acid digestibility and contribute to suboptimal growth performance of birds (Wickramasuriya *et al.*, 2015). Furthermore, the lower energy value of canola meal, when compared to soybean meal, is a drawback in the use of canola meal in high-energy poultry feeds. Nevertheless, with so much research being carried out on canola, genetic selection has successfully reduced the concentration of undesirable components such as erucic acid and glucosinolates (Leeson *et al.*, 1987; Woyengo *et al.*, 2014; Canola Council of Canada, 2015).

Encouraging the diversification of the South African poultry industry through the more economical feeding of new entrants such as the Japanese quail, helps to reduce dependency of the industry on soybean meal, address food and nutrition insecurity, and

ensure profitability. Progressive research on the physiology and production parameters of Japanese quail fed canola meal under intensive management system is scanty (Faruque *et al.*, 2013). This study, therefore, presents a comparative nutritional evaluation of canola meal as an alternative protein source for Japanese quails, a recent entrant into South African poultry industry. The objective of the study was to investigate the effect of graded levels of canola meal, as partial replacement for soybean meal, on growth performance, haematology, serum biochemistry, and meat quality. It was, therefore, hypothesised that canola-based diets promote similar performance, in terms of growth, health and meat quality in Japanese quails, to the soybean-based positive control diet.

3.2 Material and methods

3.2.1 Ethics statement

The procedures used to rear and slaughter quails were reviewed and approved by the Animal Research Ethics Committee, North West University (AREC-MC) (approval no. NWU-00521-16-A9). For the entire duration of the study, all efforts were made to minimise animal suffering (Appendix 7.2).

3.2.2 Description of the study site

The feeding trial was conducted at the North-West University Research Farm (Molelwane) with geographical coordinates of 25°40.459' S, 26°10.563' E, in the North West province of South Africa. The ambient temperature around the area ranges from 27°C to 37°C during summer, and from –3°C to 25°C in winter. The annual rainfall ranges between 300 to 600 mm. Canola meal (CM) was supplied by Southern Oil Proprietary Limited ((PTY)

Ltd), Western Cape, South Africa. While, soybean meal (SBM) was supplied by Opti feeds (PTY) LTD, North West, South Africa.

3.2.3 Soybean and canola oil extraction process

Soybean oil extraction process involved the use of isopropanol as a solvent and desolvetization at 105°C for 12 h. The canola cake meal was produced by extracting oil from the seeds harvested from canola plant following a 4-step procedure (Table 3.1) from Southern Oil (PTY) LTD. Hexane was used as the washing solvent and the desolvetizing process was intended to remove the hexane from the cake.

Table 3.1. A 4-step procedure for the oil extraction process from canola cake

Oil extraction steps	**Extraction procedure**
First Step:	*Expelling*
Moisture of seed:	7%
Oil content:	42%
Temperature during Expelling:	±90–110°C
Second Step:	*Solvent washing*
Moisture of cake (meal):	4%
Oil content:	18%
Temperature:	55°C
Third step:	*Desolvetizing*
Moisture:	±15%
Oil content:	±1.5%
Temperature:	110°C
Fourth step:	*Cooling and drying*
Moisture of cake (meal):	±11%
Oil content:	±1–2 %
Temperature:	30°C

3.2.4 Diet formulation

Five diets were formulated by replacing soybean meal (SBM) in a commercial grower diet using Format® (Optifeeds (PTY) LTD, Lichtenburg, South Africa) with graded levels of canola meal (CM). The isonitrogenous and isoenergetic experimental diets were formulated by replacing the SBM component with CM as follows: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal, producing 5 dietary treatments as shown in Table 3.2.

Table 3.2. Ingredient composition (g/kg) of canola meal-based diets

	[1]Diets				
	CON	CM25	CM50	CM125	CM175
Canola oil cake	0	25	50	125	175
Yellow maize-fine	698.6	686.9	670.2	618.2	595.1
Prime gluten 60	18.0	13.0	10.3	20.0	24.3
Full fat soya meal	50.7	71.7	104.0	185.0	174.0
Soybean meal	196.7	168.0	130.7	19.3	0.0
Limestone powder	14.5	14.2	13.8	12.8	12.2
Mono calcium phosphate	7.2	7.0	6.8	6.1	5.6
Salt-fine	3.2	3.2	3.3	3.2	3.2
Sodium bicarbonate	1.7	1.6	1.6	1.6	1.6
Choline powder	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.8	2.8	2.9	2.9
L-Threonine	0.4	0.4	0.4	0.2	0.0
Methionine	1.9	1.8	1.8	1.4	1.8
Grower-phytase	1.7	1.7	1.7	1.7	1.7
Coxistac	0.5	0.5	0.5	0.5	0.5
Olaquindox	0.4	0.4	0.4	0.4	0.4

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.

3.2.5 Chemical analyses

3.2.5.1 Proximate analyses

The formulated diets (CON, CM25, CM50, CM125 and CM175) were milled (Polymix PX-MFC 90 D) to pass through a 1 mm sieve for chemical analyses. For laboratory dry matter (AOAC, 2005: method no. 930.15) determination, approximately 1 g of each leaf sample was placed into pre-weighed crucibles and placed in an oven set at 105°C for 12 hours. The loss in weight was measured as moisture content and DM was calculated as the difference between the initial sample and moisture weights. Organic matter content (AOAC, 2005: method no. 924.05) was determined by ashing the dried samples in a muffle furnace set at 600°C for 12 hours. The loss in weight was measured as organic matter (OM) content and the residue as ash. Total nitrogen content was determined by the standard macro-Kjeldahl method (AOAC, 2005: method no. 984.13) and was converted to crude protein by multiplying the percentage N content by a factor of 6.25. Amino acids determination was conducted by hydrolysing the samples with 6 M HCl (containing phenol) for 24 h at 110 ± 2°C in glass tubes sealed under vacuum as described by Ravindran *et al.* (2005). Crude fiber was determined using the ANKOM^{2000} Fibre analyser (ANKOM Technology, New York) with 0.255 N crude fiber acid solution and then with 0.313 N crude fiber base solution. Crude fat and metabolisable energy (ME) contents were predicted using the near infrared reflectance spectroscopy (NIRs) SpectraStar XL (Unity Scientific, Australia).

3.2.5.2 Mineral analyses

Mineral contents (calcium (Ca), phosphorus (P), sodium (Na), chlorine (Cl) and potassium (K)) were analysed in the Animal Health laboratory using the dry ashing macro and micro minerals methods, following the guidelines provided by the Agri-Laboratory Association

of Southern Africa (AgriLASA, 1998). Samples that were used to determine the DM were further incinerated in a muffle furnace for 12 h. The ash was weighed and digested with 1 mL of 55% nitric acid and 10 mL of 32% hydrochloric acid using a Microwave Reaction System Model 3000. Samples were digested for 45 minutes, cooled, and transferred into respective volumetric flasks (100 mL), which were eventually topped-up with distilled water and left standing for 24 h to allow the sediment to settle down. After 24 h, samples were slowly transferred to McCartney bottles without disturbing the sediment. The concentrations of Ca, P, Na, Cl and K were then determined using an ICP Mass Spectrometer (Perkin-Elmer, NexION 300Q).

3.2.6 Experimental design

A total of 300 one-week old mixed gender Japanese quails were purchased from A & J Services Farm in Palmietfontein (Limpopo, South Africa). The quails were reared using a starter-mash purchased from Optifeeds (PTY) LTD (Lichtenburg, North West province, South Africa) and had access to fresh water with heating being provided via infrared lamps (until three weeks of age) to provide warmth. At three weeks of age, the quails were then reared using a commercial grower-mash diet (Optifeeds (PTY) LTD, Lichtenburg) until 5 weeks of age to allow differentiation of gender. At five weeks of age, a total of 140 female Japanese quails were randomly allocated to 20 replicate pens (experimental units), with each pen carrying seven female birds. The pens were in a form of standing cages with 4 partitions (pens). The size of each pen was 100 cm long × 60 cm wide × 30 cm high. The five dietary treatments were randomly allocated to the pens and the quails were reared until they were 10 weeks old. The quails were allowed to adapt to the pens and diets for a week before the experiment commenced. The experimental unit was the pen holding seven Japanese quails each, which was replicated four times per diet.

3.2.7 Feeding and quail management

Dietary treatments and clean water were provided *ad libitum* during the feeding trial. Average weekly feed intake (AWFI) per bird was measured from 6 to 10 weeks of age by subtracting the weight of the feed refused from that of the feed offered, and dividing the difference by the total number of quails in the pen. The initial live-weights of the quails were measured at the beginning of the experiment. Thereafter, average live-weight was measured weekly by weighing all the quails in each pen. These live-weights were used to calculate the average weekly weight gain (AWG) per bird as follows:

$$AWG(t_0,T) = W(T) - W(t_0),$$

where t_0 = initial time (days); T = final time; W(T) = final body weight/bird (g), and $W(t_0)$ = initial body weight/bird (g). Weekly gain: feed ratio was calculated as average weekly weight gained divided by average weekly feed consumption per bird.

3.2.8 Slaughter procedures

At 10 weeks of age, all quails were taken to Rooigrond poultry abattoir (North West province, South Africa) for slaughter. According to Berg and Raj (2015), all the quails were gas stunned by exposing them to relatively low concentrations of carbon dioxide (< 40% by volume in air), and then, once they were unconscious, exposed to a higher concentration (approximately 80% to 90% by volume in air). At the abattoir, all the quails were live-hanged onto a movable metal rack that holds them upside down by their feet. Quails were then slaughtered by cutting the jugular vein with a sharp knife and they were left hanging until bleeding stopped.

3.2.9 Blood collection and analyses

During bleeding about four mL of blood were collected using syringes from two quails randomly selected from each pen into two sets of sterilised tubes. Purple-top tubes containing ethylene diamine tetra acetic acid as an anti-coagulant were used for haematology and red-top tubes without anticoagulant were used for serum biochemical analysis. For haematology the tubes were stored in a cooler box with ice packs whereas for serum biochemistry samples were stored at room temperature for a maximum of 45 minutes to clot and thereafter refrigerated at 4°C (Washington & van Hoosier, 2012). All analyses were conducted within 48 h after collection (Buetow *et al.*, 1999). Haematological parameters (erythrocytes, haemoglobin, haematocrit, mean corpuscular volume (MCV), and mean corpuscular haemoglobin (MCH)) were determined using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc.). Mean corpuscular haemoglobin concentration (MCHC) was calculated as the ratio between the mean corpuscular haemoglobin (MCH) and the mean corpuscular volume (MCV). Clotted blood (collected in red-top tubes) was centrifuged in a micro-centrifuge at 1000 g for 15 minutes to generate serum for biochemical analysis as guided by Buetow *et al.* (1999). Albumin, alkaline phosphatase (ALP), alanine transaminase (ALT), amylase, blood calcium, serum cholesterol, creatinine, globulin, blood glucose, lipase, blood phosphorus, total bilirubin, total protein and urea were analysed using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc.).

3.2.10 Weights of internal organs and carcass traits

Upon completion of bleeding the quails were put in a de-feathering machine. Afterwards, carcasses were immediately taken to the Animal Science laboratory of the North-West University for carcass measurements and determination of meat quality parameters.

Weights of the livers, clean gizzards, hearts, wings, thighs and length of the small intestines were measured. Warm carcass weights (WCW) were measured before the carcasses were chilled for 24 h to acquire the cold carcass weight (CCW). The dressing percentages was determined as the proportion of WCW on slaughter weights.

3.2.11 Meat quality measurements

3.2.11.1 Meat pH and temperature measurements

Meat pH and temperature were recorded immediately after slaughter and 24 h post slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) according to Stanford *et al.* (2003). After every 20 measurements, the pH meter was calibrated with pH 4, pH 7 and pH 10 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland).

3.2.11.2 Meat colour

Colour of the meat (L^* = Lightness, a^* = Redness and b^* = Yellowness) was determined, immediately after slaughter and also 24 hours after slaughter, using a Minolta colour-guide (BYK-Gardener GmbH, Geretsried, Germany), on a 20 mm diameter measurement area and illuminant D65-day light, 10-degree observation angle. The colour meter was calibrated using the green standard before measurements. Colour recording was done on the surface of the thigh muscle, which was allowed to bloom for 1 hour on a polystyrene tray at 4°C. Hue angle was calculated as $\tan(\theta) = \frac{a^*}{b^*}$, and chroma was calculated as $\sqrt{a^{*2} + b^{*2}}$ as guided by Priolo *et al.* (2002).

3.2.11.3 Cooking losses and meat tenderness

After weighing, breast samples were placed in an oven set at 130°C for 20 min for determination of cooking losses. The following formula was employed:

$$Cooking\ losses\ (\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100$$

After determination of cooking losses, breast samples were sheared perpendicular to the fibre direction using a Meullenet-Owens razor shear blade mounted on an Universal Instron apparatus (cross head speed = 200 mm / minute, one shear in the centre of each core). The reported value represented the average positive peak force measurements of each sample.

3.2.12 Statistical analysis

All reported parameters were tested for normality using the NORMAL option in the Proc Univariate statement before being subjected to analysis of variance. Average weekly feed intake, average weekly weight gain and weekly gain: feed ratio data were analysed using the repeated measures analysis (SAS, 2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk},$$

where Y_{ijk} = dependant variable, μ = population mean, D_i = effect of diets, W_j = effect of week, $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ik} = random error associated with observation *ik*, assumed to be normally and independently distributed.

Overall feed intake, weight gain, gain: feed ratio, blood parameters, carcass characteristics and meat quality data were analysed using the general linear model procedure of SAS (2010). The linear statistical model employed was as follows:

$$Y_{ik} = \mu + D_i + E_{ik} ,$$

where *Yik* = dependant variable, μ = population mean, *Di* = effect of diets, and *Eik* = random error associated with observation *ik*, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P < 0.05$. Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

3.3 Results

All experimental diets were isoenergetic and isonitrogenous. Table 3.3 shows that the DM and OM content of experimental diets tended to increase as canola levels increased. Highest inclusion of canola (CM175) generally resulted in higher crude fibre and crude fat content in the diets. Phosphorus levels also tended to increase with graded levels of canola meal.

Table 3.3. Chemical composition of experimental diets on an as fed basis (g/kg, unless otherwise stated).

	[1]Diets				
	CON	CM25	CM50	CM125	CM175
Proximate components					
Dry Matter	88.65	88.71	88.82	89.14	89.06
Organic Matter	83.84	83.88	83.98	84.32	84.22
[2]ME (MJ/Kg)	12.10	12.10	12.10	12.10	11.80
Crude protein	18.00	18.00	18.01	18.61	18.94
Crude fat	4.162	4.830	5.363	6.725	6.244
Crude fibre	2.315	2.589	2.892	3.726	4.176
Minerals					
Calcium	0.850	0.850	0.850	0.850	0.850
Phosphorus	0.497	0.506	0.515	0.542	0.563
Sodium	0.180	0.180	0.180	0.180	0.180
Chlorine	0.300	0.300	0.300	0.300	0.300
Potassium	0.763	0.763	0.764	0.740	0.733
Amino acids					
Lysine	1.079	1.085	1.091	1.105	1.110
Methionine	0.478	0.476	0.475	0.460	0.520
Threonine	0.705	0.710	0.715	0.729	0.733
Tryptophan	0.187	0.189	0.192	0.197	0.201
Isoleucine	0.739	0.733	0.731	0.744	0.750
Arginine	1.102	1.101	1.102	1.101	1.100
Leucine	1.692	1.662	1.637	1.706	1.728
Valine	0.844	0.847	0.850	0.888	0.908

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]ME: metabolisable energy.

Repeated measures analysis showed no significant (P >0.05) week × diet interaction effect on AWFI, AWG and GFR. Diets significantly affected AWFI in weeks 7 and 8, but not in weeks 9 and 10 (Table 3.4) with diet CM175 promoting the least AWFI in week 7 (188.1 g/bird) and week 8 (176.4 g/bird).

Table 3.4. Average weekly feed intake (g/bird), average weekly weight gain (g/bird) and weekly gain: feed ratio (GFR) in Japanese quails fed graded levels of canola meal

	[1]Diets					[2]SEM	[3]Significance
	CON	CM25	CM50	CM125	CM175		
Feed intake (g/bird)							
Week 7	197.8^{ab}	198.3^{ab}	221.9^{b}	219.4^{b}	188.1^{a}	6.20	**
Week 8	198.3^{ab}	197.0^{ab}	215.6^{b}	212.7^{b}	176.4^{a}	8.30	*
Week 9	216.8	212.7	239.1	212.9	201.3	9.09	NS
Week 10	221.1	207.4	243.0	225.3	203.5	9.38	NS
Weight gain (g/bird)							
Week 7	39.68	41.31	46.66	40.25	41.06	4.608	NS
Week 8	18.89	19.37	19.07	21.79	13.50	3.092	NS
Week 9	9.00	6.58	2.85	8.62	6.17	1.862	NS
Week 10	7.93	4.80	5.91	1.32	5.60	2.609	NS
Gain: feed ratio							
Week 7	0.201	0.208	0.209	0.184	0.217	0.019	NS
Week 8	0.095	0.099	0.089	0.103	0.075	0.015	NS
Week 9	0.041	0.031	0.012	0.041	0.030	0.0072	NS
Week 10	0.036	0.026	0.025	0.005	0.027	0.012	NS

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]SEM: Standard error of the mean.
[3]Significance: NS = not significant; * = P <0.05; ** = P <0.01.
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

However, diet CM50 promoted the highest AWFI in week 7 (221.9 g/bird) and week 8 (212.7 g/bird). Table 3.4 shows that there were no significant differences in AWG and GFR across all weeks. There was a significant dietary effect on overall feed intake (P

<0.05). Table 3.5 shows that quails offered CM50 had higher overall feed intake (919.55 g/bird) than those offered CM175 (769.3 g/bird).

The quails on CM50 had the same (P >0.05) overall feed intake as those on CON, CM25 and CM125. However, quails on CM175 did not significantly differ with those on diets CON, CM25 and CM125 in terms of overall feed intake. There was no dietary effect (P >0.05) on overall weight gain and GFR for the entire duration of the study.

Table 3.5. Overall feed intake (g/bird), weight gain (g/bird) and gain: feed ratio (GFR) of Japanese quails fed graded levels of canola meal

	[1]Diets						
	CON	CM25	CM50	CM125	CM175	[3]SEM	[4]Significance
Overall FI (g/bird)	833.9[ab]	815.3[ab]	919.6[b]	870.2[ab]	769.3[a]	27.35	*
Overall gain (g/bird)	73.25	72.05	74.49	69.82	66.33	5.249	NS
[2]Overall GFR	0.361	0.361	0.335	0.321	0.349	0.024	NS

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]Overall GFR = overall gain: feed ratio (weekly weight gain (g/bird) / weekly feed consumed (g/bird)).
[3]SEM: Standard error of the mean.
[4]Significance: NS = not significant; * = P <0.05.
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

For haematological parameters, Table 3.6 shows that diet had no significant effect on erythrocytes (3.45–4.05 $\times 10^{12}$/L), haemoglobin (13.03–15.71 g/dL), haematocrit (0.50–0.56 L/L), mean corpuscular volume (138.36–148.84 fL), mean corpuscular haemoglobin (36.9–39.83 pg), and mean corpuscular haemoglobin concentration (26.51–28.18 g/dL) of Japanese quails.

Table 3.6. The effect of graded inclusion levels of canola meal on haematological parameters of 10-week old Japanese quails

	[1]Diets						
[2]Parameters	CON	CM25	CM50	CM125	CM175	[3]SEM	[4]Significance
Erythrocyte count ($\times 10^{12}$/L)	3.53	3.45	3.79	3.65	4.05	0.051	NS
Haemoglobin (g/dL)	13.03	13.68	14.98	13.87	15.71	0.052	NS
Haematocrit (L/L)	0.5	0.51	0.54	0.53	0.56	0.267	NS
MCV (fL)	140.1	148.8	141.7	139.4	138.4	0.413	NS
MCH (pg)	36.9	39.83	39.63	37.67	38.59	0.278	NS
MCHC (g/dL)	26.51	26.89	28.03	27.12	28.18	0.137	NS

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]Parameters: MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration.
[3]SEM: Standard error of the mean.
[4]Significance: NS = not significant.
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

With the exception of alkaline phosphatase (ALP), all biochemical parameters were not (P > 0.05) influenced by experimental diets (Table 3.7). Quail fed with CM25 had higher ALP (161.0 U/L) when compared to those on CON (37.3 U/L). The quails on diet CON had the same (P >0.05) ALP levels as those on CM50, CM125 and CM175. The birds offered CM25 did not significantly differ with those offered diets CM50, CM125 and CM175 in terms of ALP content.

Table 3.7. The effect of experimental diets on serum biochemical parameters of 10-week old Japanese quails

Parameters	[1]Diets					[2]SEM	[3]Significance
	CON	CM25	CM50	CM125	CM175		
Albumin (g/L)	22.0	22.4	18.1	20.0	23.9	1.84	NS
Alanine phosphatase (U/L)	37.3[a]	161.0[b]	111.1[ab]	112.9[ab]	81.8[ab]	23.5	**
Alanine transaminase (U/L)	41.1	50.3	44.5	37.4	40.8	11.2	NS
Amylase (U/L)	588.4	658.4	479.7	557.1	594.0	125.3	NS
Calcium (mmol/L)	2.45	2.98	2.69	2.50	2.41	0.206	NS
Cholesterol (mmol/L)	4.92	3.93	3.55	3.10	3.34	0.691	NS
Creatinine (µmol/L)	11.6	12.4	19.4	15.0	16.4	3.676	NS
Globulin (g/L)	30.0	32.3	25.1	27.7	31.0	2.591	NS
Glucose (mmol/L)	3.85	2.05	3.19	1.50	3.23	1.099	NS
Lipase (U/L)	234.9	266.0	255.3	310.3	296.5	43.0	NS
Phosphorus (mmol/L)	4.77	5.20	5.13	5.20	5.15	0.182	NS
Total bilirubin (µmol/L)	53.4	59.0	24.3	33.4	65.8	12.82	NS
Total protein (g/L)	52.0	54.7	43.3	47.7	54.9	4.318	NS
Urea (mmol/L)	1.28	1.74	1.23	1.63	1.85	0.347	NS

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]SEM: Standard error of the mean.
[3]Significance: NS = not significant; ** = P <0.01.
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

Table 3.8 shows that there were no significant dietary effects on the size of internal organs (gizzards (3.02–3.24 g), hearts (1.56–1.81 g) and livers (3.70–4.51 g)) of quails, except for length of small intestines (P <0.001). Quails on CM50 had the longest small intestines

(18.57 cm), which did not differ (P >0.05) with those on CON (17.4 cm). However, the size of small intestines in quails fed CON was similar (P >0.05) to those fed CM25, CM125 and CM175. Carcass characteristics (wings (9.06–9.75 cm), thighs (3.70–3.91 cm), WCW (117.0–151.0 g), CCW (116.1–147.4 g)), and dressing percentage (54.8-61.7%) were not influenced by experimental diets (P >0.05).

Table 3.8. Internal organs, carcass characteristics and dressing percentage of 10-week old Japanese quails fed graded levels of canola meal

	[1]Diets						
	CON	CM25	CM50	CM125	CM175	[4]SEM	[5]Significance
Small intestines (cm)	17.4[ab]	16.28[a]	18.57[b]	16.36[a]	16.95[a]	0.36	***
Wings (cm)	9.61	9.06	9.75	9.09	9.56	0.26	NS
Thighs (cm)	3.91	3.80	3.85	3.73	3.70	0.14	NS
Gizzards (g)	3.13	3.02	3.17	3.24	3.10	0.22	NS
Hearts (g)	1.56	1.81	1.56	1.80	1.60	0.16	NS
Livers (g)	4.51	4.23	4.50	4.18	3.70	0.37	NS
[2]WCW (g)	137.0	117.0	145.3	125.5	151.0	11.76	NS
[3]CCW (g)	134.6	116.1	143.7	123.3	147.4	11.39	NS
Dressing %	55.7	54.8	60.4	58.6	61.7	3.025	NS

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]WCW = warm carcass weight.
[3]CCW = cold carcass weight.
[4]SEM: Standard error of the mean.
[5]Significance: NS = not significant; *** P <0.001.
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

Table 3.9 shows that for meat quality parameters measured immediately after slaughter, experimental diets had no significant effect on meat pH (6.76 – 7.64), meat temperature (26.14 – 27.54 ℃), *L** (49.85 – 52.27), *a** (0.92 – 2.14), *b** (9.95 – 10.83), chroma (9.87 – 10.91) and hue angle (1.36 – 1.48) of female Japanese quails.

Table 3.9. The effect of graded levels of canola meal on meat quality parameters of 10-week old Japanese quails immediately after slaughter

	[1]Diets						
	CON	CM25	CM50	CM125	CM175	[2]SEM	[3]Significance
Meat pH	7.64	6.8	6.76	7.22	7.32	0.355	NS
Temperature (℃)	26.14	26.51	26.56	27.54	26.26	0.219	NS
*L**	51.37	50.91	49.85	52.27	51.25	0.796	NS
*a**	1.28	2.14	1.13	1.35	0.92	0.312	NS
*b**	10.43	9.95	9.78	10.75	10.83	0.398	NS
Chroma	10.52	10.28	9.87	10.87	10.91	0.385	NS
Hue angle	1.45	1.36	1.45	1.45	1.48	0.032	NS

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]SEM: Standard error of the mean.
[3]Significance: NS = not significant.
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

With the exception of meat pH and chroma, all meat quality parameters measured 24 h post slaughter were not significantly affected by dietary treatments (Table 3.10). Quails on CM25 had similar (P >0.05) meat pH as those on CON and CM175. Birds offered CM50 and CM125 had the same (P >0.05) meat pH as those offered CON and CM175. Meat from quails offered diet CM25 had higher chroma (7.39) than those on diet CM125 (3.58). However, CM25 quails did not differ (P >0.05) with those on CON, CM50 and CM175 in terms of meat chroma.

Table 3.10. The effect of graded levels of canola meal on meat quality parameters of 10-week old Japanese quails 24 hours after slaughter

	[1]Diets						
	CON	CM25	CM50	CM125	CM175	[2]SEM	[3]Significance
Meat pH	6.48[ab]	6.56[b]	6.38[a]	6.39[a]	6.43[ab]	0.035	**
Temperature (°C)	16.83	19.83	18.76	17.93	18.55	0.297	NS
*L**	50.89	49.35	48.23	49.36	49.49	1.036	NS
*b**	5.48	6.79	4.13	3.09	4.01	0.707	NS
*a**	2.4	2.71	2.63	1.62	2.16	0.437	NS
Chroma	6.06[ab]	7.39[b]	5.12[ab]	3.58[a]	4.72[ab]	0.835	*
Hue angle	1.1	1.2	1.02	1.04	0.97	0.09	NS

[1]Diets: CON = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal, CM50 = control diet in which 5% of soybean meal was replaced with canola meal, CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal, and CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal.
[2]SEM: Standard error of the mean
[3]Significance: NS = not significant; * = $P < 0.05$; ** = $P < 0.01$.
[a,b] In a row, dietary treatment means with common superscripts do not differ ($P < 0.05$).

Dietary treatments had no influence ($P > 0.05$) on cooking losses and peak positive force (Figure 3.1). Cooking losses ranged from 16.63–21.07 %, whereas the peak positive force ranged from 4.69–5.62 N.

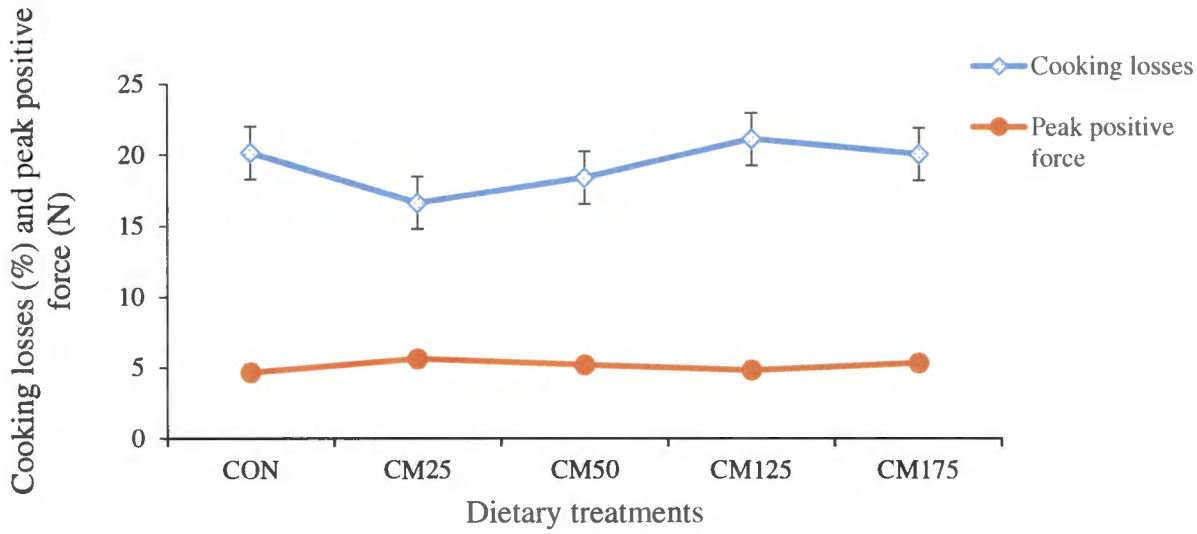

Figure 3.1. The effect of graded canola inclusion levels on cooking losses (%) and peak positive force (N)

3.4 Discussion

Determination of growth performance, haematological and biochemical parameters of quails is essential in order to evaluate the effectiveness of diets in optimising bird performance without compromising their health. Including canola meal in poultry diets at higher levels may be necessary to ensure adequate digestible amino acids (Khosravi *et al.*, 2016). However, in this study the highest inclusion level of canola (CM175) was seen to reduce AWFI, which could be a result of higher amounts of fibre content and non-starch polysaccharides in the diet, in line with the findings of Naseem *et al.* (2006). However, the negative effect on feed intake is not supported by the findings of Rojas *et al.* (1985) and Leeson *et al.* (1987), who reported that canola inclusion of 15–20% had no adverse effects on chickens. However, it is important to note the difference in bird species used in these studies since the digestive capacity of chickens and quails may differ when challenged with canola meal. Several studies have indicated that increased fibre levels reduce voluntary intake, particularly for simple non-ruminants such as quails, which have limited

ability to utilise fibrous diets (Sariçiçek *et al.*, 2005). Campbell and Slominski (1991) reported that ingestion of glucosinolates can lead to reduced feed intake and liver damage. However, it is important to note that there was no evidence of toxicity as measured by liver size and enzymes suggesting that any antinutritional factors present were not systemically harmful to the quails. Indeed, quails had similar liver weights and alanine transaminase (ALT) across all experimental diets. This could be because the concentration of glucosinolates in canola have been reduced to very low levels through genetic selection (Campbell & Slominski, 1991). The phytic acid in CM does not only interfere with phosphorus digestibility but also reduces calcium availability (Summers *et al.*, 1988) and this could be the reason why quails offered CM175 had generally low blood calcium levels. Quails had similar blood protein concentration, this was expected since experimental diets were isonitrogenous and also blood protein is not affected by partial changes of protein in the diet (Bovera *et al.*, 2007). Diets also had no significant impact on serum albumin, this was not surprising as there is a strong relationship between total protein and albumin (Omidi & Ansari nik, 2012). Bovera *et al.* (2007), reported that blood urea levels are elevated by increased dietary crude protein, which explain why diets had no significant effect on blood urea, since diets where isonitrogenous. High blood phosphorus in the diets treated with canola can be explained by the inclusion of phytase enzyme, which breaks down phytate that binds more than 60% of phosphorus and makes it unavailable for utilisation by the quail (Selle & Ravindran, 2007). The control diet was shown to generally promote high levels of blood cholesterol when compared to canola-based diets, suggesting that feeding canola to quails can reduce cholesterol levels in meat. Esonu *et al.* (2001) reported that haematological constituents demonstrate a physiological response of birds to internal and external environments, which include the type of feed and behavioural feeding patterns. However, in this study diet had no significant influence on

haematological parameters, which fell within the normal ranges for quails (Ali *et al.*, 2012). This suggests that inclusion of canola in quail diets does not negatively influence the physiological status of the birds. Also noteworthy is the fact that serum creatinine levels were similar across experimental diets indicating that including canola in quail diets has no negative effect on kidney function. The longest small intestines were observed in quails fed the control diet CON and CM50, which was surprising given that longer small intestines can be an adaptive mechanism to deal with increased amount of fibre, as longer small intestines were expected in quails fed higher levels of CM. Meat quality parameters immediately after slaughter were not influenced by dietary treatments. However, 24 hours post slaughter, diets were shown to influence meat pH, suggesting that meat pH changes with storage time. Diets had no influence on peak positive force, suggesting that substituting SBM with canola did not negatively affect meat texture.

3.5 Conclusions

This study revealed that canola meal can be a potential replacement of soybean meal in Japanese quails' diets since growth performance, physiological status and meat quality parameters were not negatively affected when canola meal was included. However, precautions may need to be taken when high amounts of canola are used, given that the highest (175 g/kg) canola inclusion level was shown to promote the least feed intake in quails. It was hypothesized that the use of feed additives such as enzymes may improve the utilization of canola meal in quails allowing its inclusion at higher levels. This potential strategy to optimize canola meal as a major protein source for quails is explored as the next step in this thesis.

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4 CHAPTER FOUR - EFFECT OF ADDING A CARBOHYDRASE MULTI-ENZYME TO CANOLA-BASED DIETS ON GROWTH PERFORMANCE, HAEMO-BIOCHEMISTRY, CARCASS AND MEAT QUALITY TRAITS OF FEMALE JAPANESE QUAILS

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Abstract

Due to the presence of non-starch polysaccharides (NSPs), canola meal (CM) tends to be of relatively lower digestibility and promote lower feed intake compared to soybean meal (SBM) and thus animals fed CM may benefit from treatment of CM-based diets with carbohydrase feed enzymes. In an internally controlled environment, a feeding trial using 6-week old, 210 female Japanese quails (189.63 ± 11.891 g live-weight) was, therefore, conducted to evaluate the effect of including a carbohydrase multi-enzyme (*endo*-1.4-beta-xylanase (> 1–< 3%; 5600 TXU/g, EC no: 232-800-2) and *endo*-1.4-beta-glucanase (> 0.3–< 1%; 2500 TGU/g, EC no. 232-734-4)) in canola-based diets on growth performance, haemo-biochemical parameters, carcass characteristics and meat quality traits of quails. Five isocaloric and isonitrogenous experimental diets were formulated as follows: control diet (CON; a commercial growers diet with no canola meal (CM) inclusion), control diet in which 17.5% of soybean meal was replaced with CM (CM0), and CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, 10% or 15% (CM50, CM100 and CM150, respectively). Dietary treatments and clean water were offered *ad libitum* during the experimental period. Average weekly feed intake (AWFI) and average weekly weight gain (AWG) were used to calculate weekly gain: feed ratio (GFR). There was a significant diet × week interaction effect on AWFI indicating that the effect of the diet changed as the

quails matured. In both weeks 8 and 9, AWFI showed significant differences between diets but no differences were observed in week 7. At 9 weeks of age, all quails were humanely slaughtered for determination of haemo-biochemical parameters, carcass characteristics and meat quality traits. For the entire duration of the study, there were no significant dietary effects on overall weight gain and GFR, however overall feed intake differed (P <0.05). Quails offered diet CM100 had higher overall feed intake (686.8 g/bird) than those offered CON (591.8 g/bird). Dietary treatments had no significant influence on haematology and serum biochemical parameters of Japanese quails. Adding the carbohydrase enzyme had no significant effect on size of internal organs, carcass and meat quality traits of quails. It was, therefore, concluded that inclusion of a carbohydrase multi-enzyme did not improve the utilisation of a canola-meal based quail diet suggesting that non-starch polysaccharides and related compounds are not important antinutritional factors in canola-based diets. However, there is a possibility that utilisation of higher canola levels can be enabled through the use of other types of enzymes targeting or countering different antinutritional factors.

4.1 Introduction

Quail farming is gaining attention from farmers, entrepreneurs, and researchers all over the world (Dauda *et al.*, 2014) because it is an economically viable alternative source of animal protein for human consumption (Puspamitra *et al.*, 2014). However, feed costs remain a major challenge mainly due to the use of soybean and full-fat soybean products during diet formulations (Elagib *et al.*, 2013; Woyengo *et al.*, 2014; Beski *et al.*, 2015; Vagadia *et al.*, 2017). The demand for soybean on the world market is high due to competition between humans and animals. The resultant higher market prices of soybean suggest the need to use less expensive protein sources to feed the quails. Canola meal (36–39% CP), a by-product after oil extraction, has been identified as a possible candidate

(Wickramasuriya *et al.*, 2015). However, canola contains ANFs that are known to restrict its utilisation in the poultry industry. Secondary plant compounds such as glucosinolates, erucic acid, phytic acid, phenolics, trypsin inhibitors and non-starch polysaccharides (NSP) limit the value of canola as a major protein source in quail feed formulations. The NSPs are a component of dietary fibre, accounting close to 18% in the canola. According to Adeola & Cowieson (2011), NSPs consist of a complex cell wall structure that binds other nutrients. Quails lack the enzymes required to break down these polysaccharides and, as consequence, they have limited capacity to utilise NSPs. Xylans and glucans are the major components of NSP found in canola and they are responsible for the reduction in effective energy and nutrient utilisation by the birds. This supports the findings reported by several scholars that canola is a potential protein source but inclusion levels greater than 250 g/kg can cause detrimental effects and reduce performance in birds (Naseem *et al.*, 2006; Ahmad *et al.*, 2007; Min *et al.*, 2011). Chapter 3 indicate that CM can be used to replace 12.5% soybean in female Japanese quail diets without compromising growth performance, health and quality of meat. However, in the same study, increasing the canola inclusion level to 17.5% of soybean resulted in a reduction in feed intake. According to Choct and Annison (1990), the antinutritive effect of NSP on nutrient digestion depends on the concentration of NSP in the diet. The application of exogenous feed enzymes to complement endogenous digestive enzymes of quails may increase utilisation and digestibility of canola meal (Cowieson & Bedford, 2009; Cowieson *et al.*, 2010). Enzymes such as carbohydrases are reported to target NSPs in the cell walls (Khajali & Slominski, 2012), and decrease the anti-nutritional effects of pentosans and β-glucans, which are the major NSPs found in canola. Carbohydrase enzymes enable the hydrolysis of cell wall polysaccharides and, therefore, reduce the encapsulating effect of cell walls resulting in enhanced availability of carbohydrates, proteins and other nutrients

(Campbell & Bedford, 1992). Indeed, the inclusion of carbohydrases in other poultry species' diets has been reported to increase nutrient bioavailability by reducing nutrient excretion and improve bird performance (Choct, 2006; Romero *et al.*, 2013). Other beneficial effects of carbohydrases include modulation of intestinal microflora (Fernández *et al.*, 2000), augmentation of digestive enzymes in growing animals (Gracia *et al.*, 2003), improving access of endogenous enzymes to cell contents (Cowieson, 2005), and reduction of endogenous amino acids losses (Gao *et al.*, 2008; Cowieson & Bedford, 2009). The effectiveness of exogenous carbohydrase in quail diets is largely unknown because most research is focused on broiler nutrition. This study was, therefore, designed to investigate the effect of treating canola-based diets with variable levels of carbohydrases on growth performance, haemo-biochemical parameters, carcass and meat quality traits of Japanese quails fed a canola-based diet. It was hypothesised that adding a carbohydrase multi-enzyme (*endo*-1, 4-beta-xylanase (> 1–< 3%; 5600 TXU/g, EC no: 232-800-2) and *endo*-1, 4-beta-glucanase (> 0.3–< 1%; 2500 TGU/g, EC no. 232-734-4)) mixture to canola-based diets will enhance growth response, haemo-biochemical parameters, carcass characteristics, and meat quality traits of female Japanese quails.

4.2 Material and methods

4.2.1 Ethics statement and study site

The procedures used to rear and slaughter quails were reviewed and approved by the Animal Research Ethics Committee, North West University (AREC-MC) (approval no. NWU-00521-16-A9) as shown in Appendix 7.2. For the entire duration of the study, all efforts were made to minimise animal suffering. The feeding experiment was conducted at Molelwane farm of the North-West University as described in Chapter 3.

4.2.2 Feed ingredients

Canola meal was acquired from Southern Oil (PTY) Ltd (Western Cape, South Africa), while, SBM was supplied by Multi Agric (Edms) Bpk (North West, South Africa). The carbohydrase multi-enzyme was sourced from BASF (PTY) LTD (Gauteng, South Africa). This enzyme is a thermo-resistant NSP complex, containing *endo*-1,4-beta-xylanase (> 1–< 3%; 5600 TXU/g, EC no: 232-800-2) and *endo*-1,4-beta-glucanase (> 0.3 - < 1%; 2500 TGU/g, EC no. 232-734-4).

4.2.3 Diet formulation

Five isonitrogenous and isocaloric experimental diets were formulated using the Format® Software (Optifeeds (PTY) LTD, Lichtenburg, South Africa) as follows: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%, as shown in Table 4.1. The inclusion levels of the carbohydrase multi-enzyme were determined based on a recommended level suggested by the supplier (0.1 g/kg inclusion rate). However, this recommended application rate was for chickens. As a result, a total of 3 inclusion levels: one level below and another above the recommended inclusion level were investigated for quails.

Table 4.1. Gross composition (g/kg) of canola meal-based diets treated with a carbohydrase multi-enzyme

	Diets[1]				
	CON	CM0	CM50	CM100	CM150
Carbohydrase	0	0	0.05	0.1	0.15
Canola oil cake	0	175	175	175	175
Yellow maize-fine	698.6	595.1	595.1	595.1	595.1
Prime gluten 60	18.0	24.3	24.3	24.3	24.3
Full fat soya meal	50.7	174.0	174.0	174.0	174.0
Soybean meal	196.7	0.0	0.0	0.0	0.0
Limestone powder	14.5	12.2	12.2	12.2	12.2
Mono calcium phosphate	7.2	5.6	5.6	5.6	5.6
Salt-fine	3.2	3.2	3.2	3.2	3.2
Sodium bicarbonate	1.7	1.6	1.6	1.6	1.6
Choline powder	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.9	2.9	2.9	2.9
L-Threonine	0.4	0.0	0.0	0.0	0.0
Methionine	1.9	1.8	1.8	1.8	1.8
Grower – phytase	1.7	1.7	1.7	1.7	1.7
Coxistac	0.5	0.5	0.5	0.5	0.5
Olaquindox	0.4	0.4	0.4	0.4	0.4

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.

4.2.4 Chemical analyses

The formulated experimental diets (CON, CM0, CM50, CM100 and CM150) were analysed for Laboratory dry matter, organic matter, total nitrogen, amino acids, crude fibre, crude fat, metabolisable energy and minerals as described in Chapter 3.

4.2.5 Experimental design

A total of 400 three-week old mixed gender Japanese quails were purchased from a farm called Quail Breeders (Gauteng, South Africa). The quails were initially reared using a commercial grower-mash diet purchased from Optifeeds (PTY) LTD (Lichtenburg, North West province, South Africa) and had access to fresh water at all times. Quails were reared until 5 weeks of age to allow differentiation of gender. At 5 weeks of age, 210 female quails were attained upon gender sexing and then randomly allocated to 30 replicate pens. The experimental unit was the pen holding 7 quails each, which was replicated 6 times per diet. The pens were in a form of standing cages with 4 partitions (pens). The size of each pen was 100 cm long × 60 cm wide × 30 cm high. Female quails were allowed to adapt to the experimental diets for a week before measurements commenced. At six weeks of age, the 5 dietary treatments were randomly allocated to the pens and the quails were reared until they were 9 weeks old. This is because by the 9^{th} week the quails had stopped growing so it would have been impossible to measure weight gain.

4.2.6 Feeding and quail management

Experimental diets and fresh water were provided *ad libitum* during the 3-week experimental period. Average weekly feed intake (AWFI) per quail was measured from 6 to 9 weeks of age by subtracting the weight of the feed refused from that of the feed offered, and dividing the difference by the total number of quails in the pen. The initial

live-weights of the quails were measured at the beginning of the experiment. Thereafter, average live-weight was measured weekly by weighing all the quails in each pen. These live-weights were used to calculate the average weekly weight gain (AWG) per bird as follows:

$$AWG(t_0,T) = W(T) - W(t_0),$$

where t_0 = initial time (days); T = final time; W(T) = final body weight/bird (g), and $W(t_0)$ = initial body weight/bird (g). Weekly gain: feed ratio was calculated as average weekly weight gained divided by average weekly feed consumption per bird.

4.2.7 Blood collection and analyses

Blood collection, storage and analyses were performed as described in Chapter 3. Haematological parameters (erythrocyte count, haemoglobin, haematocrit, leucocyte count, lymphocytes, neutrophils and monocytes) were determined using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc.). While, serum biochemical parameters (amylase, glucose, lipase and triglycerides) were analysed using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc.).

4.2.8 Slaughter procedures

After nine weeks, all quails were taken to Rooigrond poultry abattoir (North West province, South Africa) for slaughter. At the abattoir all the quails were live-hanged upside down by their feet on a rail and electrically stunned. Quails were then slaughtered by cutting the jugular vein with a sharp knife and they were left hanging until bleeding stopped. Afterwards, the quails were taken to the Animal Science laboratory for measurements of carcass characteristics and internal organs, and determination of meat quality parameters.

4.2.9 Weights of internal organs and carcass traits

Weights of the livers, cleaned gizzards, hearts, warm and cold carcasses, and length of small intestines were determined as described in Chapter 3. The dressing percentage was calculated as the proportion of warm carcass weight (WCW) on slaughter weight.

4.2.10 Meat quality measurements

Meat pH, temperature, colour (L^* = Lightness, a^* = Redness and b^* = Yellowness, hue angle and chroma), cooking losses and peak positive force were measured as described in Chapter 3.

4.2.11 Statistical analysis

All reported parameters were tested for normality using the NORMAL option in the Proc Univariate statement before being subjected to analysis of variance. Weekly feed intake, weekly weight gain and weekly GFR data were analysed using the repeated measures analysis (SAS, 2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk},$$

where Y_{ijk} = dependant variable, μ = population mean, D_i = effect of diets, W_j = effect of week, $(D \times W)_{ij}$ = effect of interaction between diets and week, E_{ik} = random error associated with observation *ik*, assumed to be normally and independently distributed.

Overall weight gain, overall GFR, blood parameters, carcass characteristics and meat quality data were analysed using the GLM procedure of SAS version 9.4 (SAS, 2010) for the diets. The linear statistical model was as follows:

$$Y_{ik} = \mu + D_i + E_{ik} ,$$

where *Yik* = dependant variable, μ = population mean, *Di* = effect of diets, and *Eik* = random error associated with observation *ik*, assumed to be normally and independently distributed.

For all statistical tests, significance was declared at $P < 0.05$. Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

4.3 Results

All experimental diets were isocaloric and isonitrogenous as indicated in Table 4.2. Both DM and OM content of diets tended to increase with CM inclusion. The inclusion of CM resulted in higher crude fibre and crude fat content in the diets. Canola-based diets tended to have higher phosphorus and methionine levels than the control diet with no canola inclusion.

Table 4.2. Chemical composition (g/kg, unless otherwise stated) of canola meal-based diets treated with a carbohydrase multi-enzyme

	[1]Diets				
	CON	CM0	CM50	CM100	CM150
Proximate components					
Dry matter	88.65	89.06	89.06	89.06	89.06
Organic matter	83.84	84.22	84.22	84.22	84.22
[2]ME (MJ/kg)	12.10	11.80	11.80	11.80	11.80
Crude protein	18.00	18.94	18.94	18.94	18.94
Crude fat	4.162	6.244	6.244	6.244	6.244
Crude fibre	2.315	4.176	4.176	4.176	4.176
Minerals					
Calcium	0.850	0.850	0.850	0.850	0.850
Phosphorus	0.497	0.563	0.563	0.563	0.563
Sodium	0.180	0.180	0.180	0.180	0.180
Chlorine	0.300	0.300	0.300	0.300	0.300
Potassium	0.763	0.733	0.733	0.733	0.733
Amino acids					
Lysine	1.079	1.110	1.110	1.110	1.110
Methionine	0.478	0.520	0.520	0.520	0.520
Threonine	0.705	0.733	0.733	0.733	0.733
Tryptophan	0.187	0.201	0.201	0.201	0.201
Isoleucine	0.739	0.750	0.750	0.750	0.750
Arginine	1.102	1.100	1.100	1.100	1.100
Leucine	1.692	1.728	1.728	1.728	1.728
Valine	0.844	0.908	0.908	0.908	0.908

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]ME: Metabolisable energy.

Repeated measures analysis showed no significant (P >0.05) week × diet interaction effect on AWG and GFR. However, a significant interaction effect was observed for AWFI. Table 4.3 indicates that diets significantly affected AWFI in weeks 8 and 9 (P <0.05), but not in week 7 (P >0.05).

Table 4.3. Average weekly feed intake (g/bird), average weekly weight gain (g/bird) and weekly gain: feed ratio in Japanese quails fed graded levels of carbohydrase-treated canola-based diets

	[1]Diets						
	CON	CM0	CM50	CM100	CM150	[2]SEM	[3]Significance
Feed intake (g/bird)							
Week 7	211.6	225.5	222.8	230.2	226.7	5.436	NS
Week 8	186.8[a]	213.0[ab]	201.4[ab]	222.8[b]	198.5[ab]	7.925	*
Week 9	193.4[a]	217.1[ab]	207.0[ab]	233.8[b]	204.2[ab]	7.715	*
Weight gain (g/bird)							
Week 7	34.16	37.29	37.59	37.36	37.43	3.117	NS
Week 8	6.82	9.22	9.91	7.26	5.43	2.124	NS
Week 9	8.25	6.06	4.26	4.89	6.54	1.472	NS
Gain: feed ratio							
Week 7	0.162	0.167	0.171	0.163	0.165	0.015	NS
Week 8	0.037	0.043	0.049	0.033	0.028	0.010	NS
Week 9	0.042	0.027	0.021	0.021	0.032	0.007	NS

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]SEM: Standard error of the mean
[3]Significance: NS = not significant; * = P <0.05
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

The CON diet promoted lower AWFI in week 8 (186.8 g/bird) and week 9 (193.4 g/bird) compared to diet CM100 in week 8 (222.8 g/bird) and week 9 (233.8 g/bird). In weeks 8 and 9, diet CON did not differ (P >0.05) with CM0, CM50 and CM150 in terms of AWFI of quails. Quails on diet CM100 had similar (P >0.05) AWFI as CM0, CM50 and CM150. Table 4.3 also shows that there were no significant differences in AWG and GFR for the entire duration of the feeding trial. Table 4.4 indicates that across all weeks, there were no dietary effect (P >0.05) on overall weight gain and overall GFR.

Table 4.4. Effect of carbohydrase-treated canola-based diets on overall weight gain (g/bird) and gain: feed ratio of Japanese quails

	[1]Diets						
	CON	CM0	CM50	CM100	CM150	[3]SEM	[4]Significance
Overall gain (g/bird)	57.17	51.52	51.23	51.29	50.23	5.900	NS
[2]Overall GFR	0.277	0.233	0.234	0.224	0.230	0.031	NS

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]Overall GFR: Gain: feed ratio for the entire duration of the study.
[3]SEM: standard error of the mean.
[4]Significance: NS = not significant; * = P <0.05.
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05).

For haematological parameters, Table 4.5 indicates that diets had no significant effect on erythrocyte count (3.10–3.45 $\times 10^{12}$/L), haemoglobin (12.08–13.78 g/dL), haematocrit (0.51–0.57 L/L), leucocytes (34.72–50.73 $\times 10^{9}$/L), lymphocytes (27.65–43.23 $\times 10^{9}$/L), neutrophils (3.53–7.59 $\times 10^{9}$/L), and monocytes (1.38–2.61 $\times 10^{9}$/L) of Japanese quails.

Table 4.5. Effect of carbohydrase-treated canola-based diets on haematological parameters of 10-week old Japanese quails

	[1]Diets						
	CON	CM0	CM50	CM100	CM150	[2]SEM	[3]Significance
Erythrocyte count ($\times 10^{12}$/L)	3.34	3.21	3.10	3.45	3.27	0.206	NS
Haematocrit (L/L)	0.55	0.52	0.51	0.57	0.54	0.026	NS
Haemoglobin (g/dL)	13.68	13.21	12.08	13.78	13.76	0.709	NS
Leucocyte count ($\times 10^{9}$/L)	34.72	42.88	35.74	44.23	50.73	10.887	NS
Lymphocytes ($\times 10^{9}$/L)	27.65	32.50	30.35	37.89	43.23	8.956	NS
Monocytes ($\times 10^{9}$/L)	1.75	2.61	2.01	1.82	1.38	0.927	NS
Neutrophils ($\times 10^{9}$/L)	5.73	7.59	4.32	3.53	6.01	4.970	NS

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]SEM: standard error of the mean.
[3]Significance: NS = not significant.

For serum biochemical parameters, Table 4.6 shows that diet had no significant effect on amylase, glucose, lipase and triglyceride of Japanese quails. Serum amylase ranged from 300.4 to 512.9 IU/L, whereas lipase ranged from 54.42 to 79.92 IU/L. Glucose ranged from 15.37 to 16.45 mmol/L, whereas triglyceride ranged from 0.861 to 1.633 mmol/L.

Table 4.6. Effect of carbohydrase-treated canola-based diets on serum biochemical parameters of 10-week old Japanese quails

	[1]Diets						
Biochemical parameters	CON	CM0	CM50	CM100	CM150	[2]SEM	[3]Significance
Amylase (IU/L)	367.0	512.9	437.8	300.4	495.4	71.74	NS
Glucose (mmol/L	15.66	15.37	16.45	15.65	15.54	0.393	NS
Lipase (IU/L)	54.42	79.92	55.25	68.33	63.60	12.44	NS
Triglyceride (mmol/L)	1.633	0.861	1.015	0.953	0.928	0.255	NS

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]SEM: standard error of the mean.
[3]Significance: NS = not significant.

Table 4.7 shows that there were no significant dietary influences on internal organs, carcass characteristics and dressing percentage of the quails (P >0.05). The weights of the hearts ranged from 1.98 to 2.29 g, while those of the gizzards ranged from 3.93 to 4.40 g. Liver weights ranged from 3.95 to 4.65 g. The length of small intestines ranged from 51.2 to 54.4 cm. The WCW ranged from 143.2 to 164.3 g, whereas CCW ranged from 141.3 to 163.3 g. The dressing percentage ranged from 59.3 to 69.5%.

Table 4.7. The effect of carbohydrase-treated canola-based diets on internal organs, carcass traits and dressing percentage of 10-week old Japanese quails

	[1]Diets						
	CON	CM0	CM50	CM100	CM150	[4]SEM	[5]Significance
Hearts (g)	1.98	2.29	2.08	2.14	2.21	0.096	NS
Gizzards (g)	3.93	3.93	4.40	4.39	4.27	0.187	NS
Livers (g)	3.95	4.65	4.36	4.15	4.27	0.181	NS
Small intestines (cm)	51.2	54.4	54.0	52.5	52.9	1.732	NS
[2]WCW (g)	143.2	157.5	152.8	153.9	164.3	7.191	NS
[3]CCW (g)	141.3	156.5	151.5	152.7	163.3	7.119	NS
Dressing %	59.3	65.6	66.8	60.6	69.5	3.373	NS

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]WCW: warm carcass weight.
[3]CCW: cold carcass weight.
[4]SEM: standard error of the mean.
[5]Significance: NS = not significant.

Table 4.8 shows that experimental diets had no significant effect on meat quality parameters measured immediately after slaughter of female Japanese quails. Meat pH ranged from 5.752 to 5.947, meat temperature ranged from 22.38 to 23.67 °C, meat lightness (L^*) ranged from 46.81 to 48.36, meat redness (a^*) ranged from 1.132 to 1.463, meat yellowness (b^*) ranged from 8.742 to 9.647, and meat chroma ranged from 8.822 to 9.792 and hue angle ranged from 1.407 to 1.456.

Table 4.8. The effect of carbohydrase-treated canola-based diets on meat quality parameters of 10-week old Japanese quails immediately after slaughter

	[1]Diets						
	CON	CM0	CM50	CM100	CM150	[2]SEM	[3]Significance
Meat pH	5.817	5.752	5.943	5.947	5.863	0.073	NS
Temperature (°C)	22.57	23.62	22.38	23.67	23.42	0.609	NS
*L**	48.13	48.36	47.11	47.47	46.81	0.861	NS
*a**	1.332	1.132	1.268	1.463	1.315	0.183	NS
*b**	9.095	9.708	8.867	8.742	9.647	0.396	NS
Chroma	9.195	9.792	8.822	8.870	9.742	0.403	NS
Hue angle	1.427	1.456	1.424	1.407	1.435	0.018	NS

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]SEM: standard error of the mean.
[3]Significance: NS = not significant.

Table 4.9 indicates that for meat quality parameters measured 24 h post-slaughter, dietary treatments had no significant influence on meat pH (6.38–6.59), meat temperature (10.37–11.32 °C), *L** (46.61–47.77), *a** (2.47–3.81), *b** (11.36–11.87), chroma (11.66–12.45) and hue angle (1.26–1.36) of Japanese quails.

Table 4.9. The effect of carbohydrase-treated canola-based diet on meat quality parameters of 10-week old Japanese quails 24 h after slaughter

	[1]Diets						
	CON	CM0	CM50	CM100	CM150	[2]SEM	[3]Significance
Meat pH	6.59	6.65	6.38	6.49	6.59	0.125	NS
Temperature (°C)	10.61	11.32	10.37	10.51	10.52	0.319	NS
*L**	47.27	47.77	47.50	47.23	46.61	0.513	NS
*a**	3.74	2.47	3.34	3.81	3.47	0.348	NS
*b**	11.87	11.36	11.73	11.63	11.68	0.302	NS
Chroma	12.45	11.66	12.21	12.26	12.20	0.348	NS
Hue angle	1.27	1.36	1.29	1.26	1.28	0.025	NS

[1]Diets: CON = control diet (a commercial growers diet with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with CM, CM50 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150 = CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%.
[2]SEM: standard error of the mean.
[3]Significance: NS = not significant.

Figure 4.1 indicates that experimental diets had no influence (P >0.05) on cooking losses and peak positive force. Cooking losses ranged from 23.60 to 25.70%, while the peak positive force ranged from 5.15 to 6.80 N.

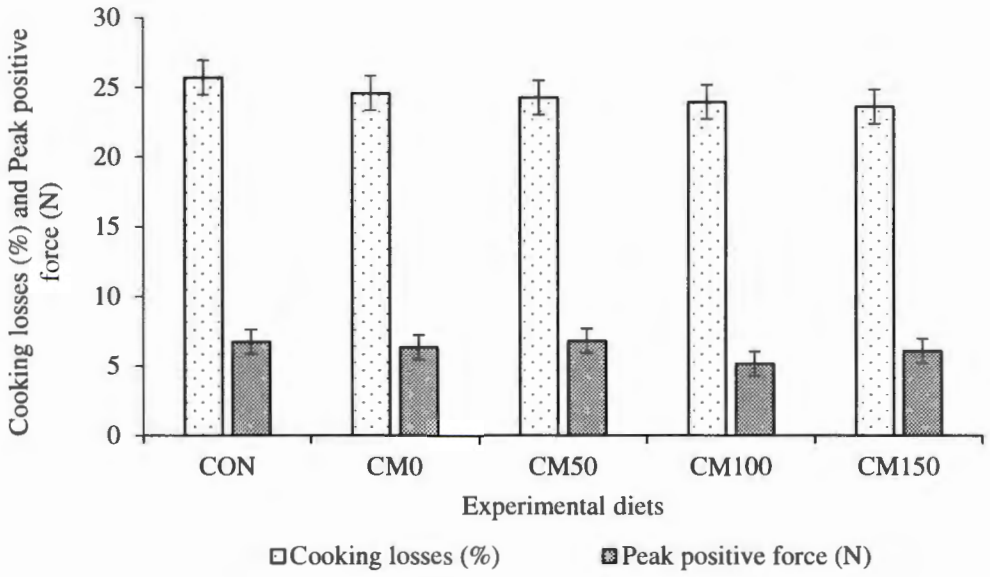

Figure 4.1. Cooking losses (%) and peak positive force (N) of Japanese quails as influenced by experimental diets

4.4 Discussion

The utility of exogenous feed enzyme supplements in canola-SBM diets for Japanese quails is largely unknown, despite the existence of a few studies reporting positive effects on physiological responses, blood parameters, and meat quality in broilers. Exogenous feed enzymes are a potential strategy through which the use of canola meal as an alternative protein source in quail diets can be optimized. According to Wickramasuriya *et al.* (2015) and Khosravi *et al.* (2016), CM has higher methionine content compared to soybean and this explains why the inclusion of CM in quail diets in this study increased methionine levels, a major limiting amino acid in birds (Canola Council of Canada, 2009). Repeated measures analysis revealed a significant diet × week interaction effect on average weekly feed intake (AWFI), signifying that the influence of the diet on feed intake changed as the quails grew older. In week 8 and week 9, the control diet promoted lower AWFI compared to diet CM100, suggesting a compensatory feeding by the quails due to dilution of nutrient concentration. This could be attributed to the increased amount of fibre in the diet, which could have affected feed intake. Considering that the inclusion level of

CM was 17.5% in place of soybean, it is likely that the NSP content in the canola would have induced a decrease on feed intake. However, addition of the carbohydrases was intended to degrade the NSP and as a consequence facilitate the absorbance of nutrients and improve weight gain. According to Walugembe *et al.* (2014) feeding fibrous diets in poultry tends to increase feed intake as a way to compensate for the reduced nutrient concentration in feed. However, according to Chapter 3 dietary inclusion of canola beyond 125 g/kg reduces feed intake and can compromise the performance of female Japanese quails.

The use of feed additives to enhance the utilisation of canola-based diets is believed to be a solution, if canola is to be included in higher amounts in order to reduce feed costs. The use of exogenous enzymes has been extensively investigated on simple non-ruminants for cost-effective feed formulations (Bedford, 2000; Adeola & Cowieson, 2011). Application of exogenous carbohydrase enzymes has been reported to improve feed utilisation, nutrient digestibility and weight gain in birds fed fibrous diets (Gracia *et al.*, 2003; Adeola & Bedford, 2004; Romero *et al.*, 2013). Single enzymes, such as xylanases and glucanases are mostly incorporated in poultry diets, representing more than 80% of the global carbohydrase market (Adeola & Cowieson, 2011). However, in this study, treating the canola-based diets did not improve feed intake, weight gain and GFR, which was in agreement with the findings of Simbaya *et al.* (1996), Meng and Slominski (2005) together with Mushtaq *et al.* (2007) who reported no effect of multi-carbohydrase enzymes in chickens fed CM-based diets. Indeed, Jia *et al.* (2012) and Radfar *et al.* (2017) also found no improvement on broilers fed diets containing 150 g/kg of CM supplemented with carbohydrase enzymes. The differences on the mode of action/ performance of the carbohydrases can be attributed to variations in application methods and the activities of the enzymes that were used in different studies (Yuan *et al.*, 2008).

Experimental diets had no significant impact on haematological and serum biochemical parameters of female Japanese quails, which fell within the normal range for quails (Ali *et al.*, 2012). This suggests that carbohydrase treatment of quail diets did not influence the physiological and pathophysiological status of quails. It was expected that supplementation with carbohydrases would reduce the activity of pancreatic digestive enzymes, which would partly be displayed as lower serum amylase activity. Triglyceride values were also within the normal ranges for quails, indicating that there were no diet-induced modifications in the energy and fat metabolism of quails. Carbohydrases-treated diets did not affect sizes of internal organs, carcass characteristics, pH, temperature, and colour of the meat, which were similar to those of quails fed untreated CM-based diet. These findings are in agreement with Gracia *et al.* (2003), who observed no effect of carbohydrase single enzyme supplementation on relative weights of broilers' organs. Longer small intestines in quails offered CM-based diets have also been observed in Chapter 3 and could be a result of an adaptive mechanism to deal with the increased amounts of fibre for efficient digestion and absorption of nutrients. Experimental diets also had no significant influence on cooking losses and peak positive force values of female Japanese quails, suggesting that application of carbohydrases did not improve these meat quality traits.

4.5 Conclusions

All reported discrepancies among authors are not clear but might be attributed to the amount of enzyme activity included in the diet. The current study revealed that carbohydrase-treated diets promoted similar performance in terms of growth response, health status and meat quality traits as the canola-based diet. It was, therefore, concluded that the inclusion of an exogenous carbohydrase multi-enzyme did not improve the utilisation of a CM based quail diet. However, there is a possibility that utilisation of

higher canola levels can be enabled through the use of other types of enzymes targeting or countering different antinutritional factors.

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5 CHAPTER FIVE - PROTEASE-TREATED CANOLA-BASED JAPANESE QUAIL DIETS: EFFECT ON PHYSIOLOGICAL PARAMETERS AND MEAT QUALITY TRAITS

Paper submitted to the Journal, Animal Nutrition

Abstract

The current study was conducted to assess the effect of adding a heat-stable protease mono-enzyme to canola-based diets on growth performance, haemo-biochemical parameters, carcass characteristics and meat quality traits of female Japanese quails (*Coturnix coturnix japonica*). A total of 240 5-week old female Japanese quails (163.85 ± 9.564 g body-weight) were randomly allocated to five dietary treatments distributed across six replicate pens with each pen carrying eight quails. The five isoenergetic and isonitrogenous dietary treatments were formulated as follows: control diet (CON; a commercial growers mash with no canola meal (CM) inclusion), control diet in which 17.5% of soybean meal (SBM) was replaced with CM (CM0), and CM0 diet treated with 10%, 20% and 30% protease enzyme (CM10, CM20 and CM30, respectively). At 10 weeks of age, all quails were humanely slaughtered. There was no significant week × diet interaction effect on average weekly feed intake (AWFI), average weekly weight gain (AWG) and gain: feed ratio (GFR). Adding protease to CM-based diets had no significant influence on haemo-biochemical parameters of the Japanese quails. There were no dietary effects (P >0.05) in terms of size of internal organs, carcass characteristics and dressing percentage. Application of protease had no influence on meat quality parameters measured immediately and 24 h after slaughter. It was concluded that the inclusion of exogenous protease enzyme does not improve the utilisation of a CM-based quail diet. It is, therefore,

recommended that the use of mixtures of different enzymes and the application of enzymes at higher inclusion levels may allow the inclusion of CM at higher levels.

5.1 Introduction

The evolution of Japanese quails in the poultry industry to complement the existing birds is driven by the high demand of dietary protein to feed the fast growing human population. Quails have superior qualities over other poultry birds (Ayaşan, 2013; Puspamitra *et al.*, 2014; Mnisi *et al.*, 2017). Provision of high quality protein feeds is necessary to meet the nutritional requirements of quails and increase their production. Soybean is one of the vegetable protein sources largely incorporated during feed formulations by food and feed manufacturing industries because of its high protein content and a relatively well-balanced amino acid profile (Cromwell *et al.*, 1999). Currently, the use of soybean meal (SBM) is limited by its high market prices, which has resulted in high feed costs. This therefore leaves researchers with no option but to seek for alternative and inexpensive protein sources such as canola. Canola meal (CM) has recently been accepted as a protein source that has the potential to partially replace SBM in poultry diets (Naseem *et al.*, 2006; Mushtaq *et al.*, 2007). Incorporation of CM in quail diets can reduce feed costs and increase flexibility in feed formulation.

Despite the fact that canola protein is a potential ingredient for use in the food industry, its utilisation is limited by the presence of some undesirable compounds such as protease inhibitors, glucosinolates, phytates, phenols, non-starch polysaccharides and high dietary fibre, which reduce its biological utilization, nutrient bioavailability and eventually decrease growth performance (Aidera & Barbanab, 2011; Singh *et al.*, 2017). Canola meal has low digestible proteins which can be related to the presence of protease/trypsin inhibitors (Hussain, 2015). Indeed, inclusion levels of CM more than 125 g/kg in place of

SBM is reported to reduce voluntary feed intake in Japanese quails (Chapter 3). This is because trypsin inhibitors are known to bind to trypsin and chymotrypsin enzymes and reduce their digestive activities.

Furthermore, quail produces proteolytic enzymes that are sufficient for protein utilization (Nir *et al.*, 1993) but a substantial amount of protein passes the gastro-intestinal tract without being completely digested (Wang & Parsons, 1998; Lemme *et al.*, 2004). This is also likely to occur when canola is fed due to the presence of protease/trypsin inhibitors (Berot *et al.*, 2005; Hussain, 2015) that interfere with the proteolytic activities of pepsin or trypsin and hinder protein/amino acid digestibility (Sariçiçek *et al.*, 2005). Exogenous proteases, therefore, provide an opportunity to improve protein and amino acid digestibility (Rostagno & Becker, 2005; Angel *et al.*, 2011). In order to increase the inclusion levels of CM in quail diets, the application of an exogenous mono-component protease enzyme is necessary to liberate digestible amino acids conjugated with xylans and starch by disrupting the starch-protein matrix (Zanella *et al.*, 1999; Wang *et al.*, 2006). The incorporation of enzymes in poultry diets is reported to increase energy utilization, nutrient digestibility and thereby enhance weight gain (Cowieson & Ravindran, 2008; Romero *et al.*, 2013; Stefanello *et al.*, 2016). Exogenous proteases were reported to improve feed efficiency and broiler performance when fed canola-based diets (Simbaya *et al.*, 1996; Liu *et al.*, 2015). Furthermore, Ghazi *et al.* (2003) reported that supplementing broilers diets with exogenous protease improved growth performance and protein utilization. The efficacy of exogenous proteases in canola-based quail diets is largely unknown as too much attention is on broiler nutrition (Ding *et al.*, 2016). This study was, therefore, designed to investigate the effect of treating canola-based diets with graded levels of protease on physiological parameters, carcass and meat quality characteristics of female Japanese quails fed a canola-based diet. We hypothesised that adding a mono-

component protease enzyme (75'000 PROT/g; EC/IUB no. 3.4.21) to canola-based diets will improve growth performance, haematological and serum biochemical parameters, carcass characteristics, and meat quality traits of female Japanese quails.

5.2 Materials and methods

5.2.1 Ethics statement

The experiment was reviewed and approved by the Animal Research Ethics Committee, North-West University (AREC-MC); approval number: NWU-00521-16-A9 (Appendix 7.2).

5.2.2 Description of study site and feed ingredients

The study was conducted at the North-West University Molelwane farm as indicated in Chapter 3. Canola meal was purchased from Southern Oil (PTY) LTD (Western Cape, South Africa). While, SBM and all other ingredients were supplied by Optifeeds (PTY) LTD (North West, South Africa). The protease enzyme (75'000 PROT/g; EC/IUB 3.4.21) was received from a feed manufacturing company called A-feeds (PTY) LTY (Gauteng, South Africa).

5.2.3 Dietary treatment formulation

Five isoenergetic and isonitrogenous dietary treatments were formulated using Format® (Optifeeds (PTY) LTD, Lichtenburg, South Africa) as follows: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme, as shown in Table 5.1. The

inclusion levels of the proteases were determined based on a recommended level prescribed by the supplier (0.2 g/kg inclusion rate). However, this recommended application rate was for chickens. As a result, a total of 3 inclusion levels: one level below and another above the recommended inclusion level were investigated for quails.

Table 5.1. Gross composition (g/kg) of canola meal-based diets treated with a mono-component protease enzyme

	[1]Diets				
	CON	CM0	CM10	CM20	CM30
Protease	0	0	0.10	0.20	0.30
Canola oil cake	0	175.0	175.0	175.0	175.0
Fine yellow maize	698.6	595.1	595.1	595.1	595.1
Prime gluten 60	18.0	24.3	24.3	24.3	24.3
Full fat soya meal	50.7	174.0	174.0	174.0	174.0
Soybean meal	196.7	0.0	0.0	0.0	0.0
Limestone powder	14.5	12.2	12.2	12.2	12.2
Mono calcium phosphate	7.2	5.6	5.6	5.6	5.6
Fine salt	3.2	3.2	3.2	3.2	3.2
Sodium bicarbonate	1.7	1.6	1.6	1.6	1.6
Choline powder	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.9	2.9	2.9	2.9
L-Threonine	0.4	0.0	0.0	0.0	0.0
Methionine	1.9	1.8	1.8	1.8	1.8
Grower – phytase	1.7	1.7	1.7	1.7	1.7
Coxistac	0.5	0.5	0.5	0.5	0.5
Olaquindox	0.4	0.4	0.4	0.4	0.4

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.

5.2.4 Chemical analyses

The formulated dietary treatments (CON, CM0, CM10, CM20 and CM30) were analysed for laboratory dry matter, organic matter, total nitrogen, amino acids, crude fibre, crude fat, metabolisable energy and minerals as described in Chapter 3.

5.2.5 Experimental design

A total of 400 three-week old mixed gender Japanese quails were purchased from a farm called Quail Breeders (Gauteng, South Africa). The quails were reared until 5 weeks of age to allow gender differentiation using a commercial grower-mash diet purchased from Optifeeds (PTY) LTD (Lichtenburg, North West province). At 5 weeks of age, a total of 240 female Japanese quails were attained upon gender sexing. The female quails were randomly allocated to 30 replicate pens. The experimental unit was the pen holding 8 quails each, which was replicated 6 times per dietary treatment. The pens were in a form of standing cages with 4 partitions (pens). The size of each pen was 100 cm long × 60 cm wide × 30 cm high. The five dietary treatments (CON, CM0, CM10, CM20 and CM30) were randomly allocated to the pens and quails were reared until they were 10 weeks old. Quails were allowed to adapt to dietary treatments for a week before measurements commenced.

5.2.6 Feeding and quail management

Experimental diets and clean water were provided *ad libitum* during the feeding trial. Average weekly feed intake (AWFI) per bird was measured from 6 to 10 weeks of age by subtracting the weight of the feed refused from that of the feed offered, and dividing the difference by the total number of quails in the pen. The initial live-weights of the quails were measured at the beginning of the experiment. Thereafter, average live-weight was

measured weekly by weighing all the quails in each pen. These live-weights were used to calculate the average weekly weight gain (AWG) per bird as follows:

$$AWG(t_0,T) = W(T) - W(t_0),$$

where t_0 = initial time (days); T = final time; W(T) = final body weight/bird (g), and $W(t_0)$ = initial body weight/bird (g). Weekly gain: feed ratio was calculated as average weekly weight gained divided by average weekly feed consumption per bird.

5.2.7 Blood collection and analyses

Blood collection, storage and analyses were performed as described in Chapter 3. Haematological parameters (erythrocyte counts, haemoglobin, haematocrit, leucocyte counts, lymphocytes, monocytes and eosinophils) were determined using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc.). Serum biochemical parameters (total protein, albumin, bilirubin, creatinine and urea) were analysed using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc.).

5.2.8 Slaughter procedures

After 10 weeks, all quails were taken to Rooigrond poultry abattoir (North West province, South Africa) for slaughter as indicated in Chapter 4. Afterwards, quails were taken to the North-West University Animal Science laboratory for determination of internal organs and carcass characteristics as well as meat quality parameters.

5.2.9 Weights of internal organs and carcass characteristics

Weights of the livers, cleaned gizzards, hearts, warm and cold carcasses, and length of small intestines were determined as described in Chapter 3. The dressing percentage was calculated as the proportion of warm carcass weight (WCW) on slaughter weight.

5.2.10 Meat quality measurements

Meat pH, temperature, colour (L^* = Lightness, a^* = Redness and b^* = Yellowness, hue angle and chroma), cooking losses and peak positive force were determined as indicated in Chapter 3.

5.2.11 Statistical analysis

All reported parameters were tested for normality using the NORMAL option in the Proc Univariate statement before being subjected to analysis of variance. Weekly feed intake, weekly weight gain and weekly GFR data were analysed using the repeated measures analysis (SAS, 2010). The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk},$$

where Y_{ijk} = dependant variable, μ = population mean, D_i = effect of dietary treatments, W_j = effect of week, $(D \times W)_{ij}$ = effect of interaction between dietary treatments and week, E_{ik} = random error associated with observation *ik*, assumed to be normally and independently distributed.

Overall feed intake, overall weight gain, overall gain: feed ratio, blood parameters, carcass characteristics and meat quality data were analysed using the GLM procedure of SAS version 9.4 (SAS, 2010) for the dietary treatments. The linear statistical model was as follows:

$$Y_{ik} = \mu + D_i + E_{ik} ,$$

where *Yik* = dependant variable, μ = population mean, *Di* = effect of dietary treatments, and *Eik* = random error associated with observation *ik*, assumed to be normally and independently distributed.

For all statistical tests, significance was declared at $P < 0.05$. Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

5.3 Results

Table 5.2 shows the chemical composition of the isoenergetic and isonitrogenous experimental diets on an as-fed basis. Inclusion of CM resulted in higher crude fibre, crude protein and crude fat content of the diets. Canola-based diets had a fairly comparable amino acid profile to the soybean-based control diet.

Table 5.2. Chemical composition (g/kg, unless otherwise stated) of canola meal-based diets treated with a mono-component protease enzyme

	[1]Diets				
	CON	CM0	CM10	CM20	CM30
Proximate components					
Dry matter	88.65	89.06	89.06	89.06	89.06
Organic matter	83.84	84.22	84.22	84.22	84.22
[2]ME (MJ/kg)	12.10	11.80	11.80	11.80	11.80
Crude protein	18.00	18.94	18.94	18.94	18.94
Crude fat	4.162	6.244	6.244	6.244	6.244
Crude fibre	2.315	4.176	4.176	4.176	4.176
Minerals					
Calcium	0.850	0.850	0.850	0.850	0.850
Phosphorus	0.497	0.563	0.563	0.563	0.563
Sodium	0.180	0.180	0.180	0.180	0.180
Chlorine	0.300	0.300	0.300	0.300	0.300
Potassium	0.763	0.733	0.733	0.733	0.733
Amino acids					
Lysine	1.079	1.110	1.110	1.110	1.110
Methionine	0.478	0.520	0.520	0.520	0.520
Threonine	0.705	0.733	0.733	0.733	0.733
Tryptophan	0.187	0.201	0.201	0.201	0.201
Isoleucine	0.739	0.750	0.750	0.750	0.750
Arginine	1.102	1.100	1.100	1.100	1.100
Leucine	1.692	1.728	1.728	1.728	1.728
Valine	0.844	0.908	0.908	0.908	0.908

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]ME: Metabolisable energy.

Repeated measures analysis indicated no significant (P >0.05) week × diet interaction effect on AWFI, AWG and GFR. Table 5.3 shows that there were no significant dietary influences on growth performances of female Japanese quails in terms of AWFI, AWG and weekly GFR (P >0.05).

Table 5.3. Average weekly feed intake (g/bird), average weekly weight gain (g/bird) and weekly GFR in Japanese quails fed graded levels of protease-treated canola-based diets

	[1]Diets						
	CON	CM0	CM10	CM20	CM30	[2]SEM	*P*-value
Feed intake (g/bird)							
Week 7	193.3	209.3	202.5	194.0	204.5	5.012	0.1414
Week 8	182.8	201.1	183.3	198.1	194.5	7.444	0.2986
Week 9	175.4	194.1	174.8	190.1	176.8	9.217	0.4342
Week 10	158.2	171.2	171.7	171.8	166.4	8.350	0.7438
Weight gain (g/bird)							
Week 7	46.77	51.17	43.89	46.81	44.13	3.809	0.6708
Week 8	20.40	24.58	20.35	21.43	21.63	2.626	0.7233
Week 9	22.81	13.85	17.35	24.05	9.00	7.800	0.4985
Week 10	2.80	2.18	6.03	9.71	8.15	3.916	0.2782
Gain: feed ratio							
Week 7	0.242	0.246	0.216	0.242	0.216	0.018	0.6097
Week 8	0.112	0.123	0.110	0.109	0.109	0.013	0.9119
Week 9	0.109	0.089	0.051	0.076	0.119	0.033	0.5534
Week 10	0.041	0.059	0.040	0.038	0.023	0.022	0.7998

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]SEM: standard error of the mean.

Table 5.4 indicates that dietary treatments had no significant effect on overall feed intake, overall weight gain and overall GFR. The overall feed intake ranged from 726.4 to 753.7 g/bird. Overall weight gain ranged from 71.35–90.98 g/bird, whereas overall GFR ranged from 0.373–0.465 (weight gain (g/bird) / feed consumed (g/bird)).

Table 5.4. Overall effect of protease-treated canola-based diets on feed intake (g/bird), weight gain (g/bird) and GFR of 10-week old Japanese quails

	[1]Diets						
	CON	CM0	CM10	CM20	CM30	[3]SEM	*P*-value
Overall FI (g/bird)	753.7	753.0	753.1	727.7	726.4	27.52	0.8931
Overall Gain (g/bird)	90.98	81.73	78.40	88.59	71.35	8.993	0.5525
[2]Overall GFR	0.465	0.403	0.397	0.462	0.373	0.0448	0.5089

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]Overall GFR: gain: feed ratio for the entire duration of the study.
[3]SEM: standard error of the mean.

Table 5.5 indicates that diets did not significantly influence haematological parameters (P >0.05) of female Japanese quails. Eosinophils ranged from 1.96 to 2.84 $\times 10^9$/L, erythrocyte count ranged from 2.91 to 3.15 $\times 10^{12}$/L, haematocrit ranged from 0.473 to 0.508 L/L, haemoglobin ranged from 11.9 to 12.5 g/dL, leucocyte count ranged from 32.0 to 41.1 $\times 10^9$/L, lymphocytes ranged from 25.0 to 37.8 $\times 10^9$/L, and monocytes ranged from 1.15 to 1.98 $\times 10^9$/L.

Table 5.5. Effect of protease-treated canola-based diets on haematological parameters of 10-week old Japanese quails

	[1]Diets						
	CON	CM0	CM10	CM20	CM30	[2]SEM	*P*-value
Eosinophils ($\times 10^9$/L)	2.68	2.72	2.21	1.96	2.84	0.986	0.941
Erythrocyte count ($\times 10^{12}$/L)	2.91	3.06	3.15	3.07	3.14	0.206	0.854
Haematocrit (L/L)	0.486	0.503	0.508	0.473	0.506	0.029	0.838
Haemoglobin (g/dL)	12.3	12.5	11.9	12.0	12.2	0.555	0.904
Leucocyte count ($\times 10^9$/L)	41.1	36.5	32.0	40.7	36.3	9.895	0.936
Lymphocytes ($\times 10^9$/L)	37.8	31.5	27.4	25.0	31.2	9.794	0.856
Monocytes ($\times 10^9$/L)	1.15	1.98	1.28	1.89	1.76	0.745	0.802

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]SEM: standard error of the mean.

Table 5.6 shows that diet had no significant effect on serum biochemical parameters of female Japanese quails. Total protein ranged from 32.4 to 37.3 g/L, whereas albumin ranged from 9.0 to 10.9 g/L. Creatinine ranged from 18.0 to 19.0 μmol/L, while bilirubin ranged from 0.626 to 1.073 μmol/L. Urea ranged from 0.408 to 0.60 mmol/L.

Table 5.6. Effect of protease-treated canola-based diets on serum biochemical parameters of 10-week old Japanese quails

	[1]Diets						
	CON	CM0	CM10	CM20	CM30	[2]SEM	*P*-value
Total protein (g/L)	37.3	32.8	32.4	35.3	36.7	5.30	0.901
Albumin (g/L)	10.6	10.0	9.3	9.0	10.9	1.55	0.843
Creatinine (µmol/L)	18.1	18.0	18.0	18.0	19.0	0.692	0.558
Bilirubin (µmol/L)	0.626	0.667	0.813	0.656	1.073	0.164	0.228
Urea (mmol/L)	0.408	0.502	0.522	0.588	0.600	0.080	0.456

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]SEM: standard error of the mean.

Table 5.7 shows that there was no dietary influence ($P > 0.05$) in terms of size of internal organs, carcass characteristics and dressing percentage. The weights of the livers ranged from 4.45 to 4.72 g, hearts ranged from 2.08 to 2.25 g and gizzards ranged from 4.14 to 4.40 g. The length of small intestines ranged from 50.57 to 55.61 cm. Cold carcass weights ranged from 164.6 to 176.5 g, whereas the WCW ranged from 168.8 to 176.9 g. The dressing percentage ranged from 67.92 to 69.97%.

Table 5.7. The effect of protease-treated canola-based diets on size of internal organs, carcass traits and dressing percentage of 10-week old female Japanese quails

	[1]Diets						
	CON	CM0	CM10	CM20	CM30	[4]SEM	*P*-value
Livers (g)	4.45	4.69	4.72	4.48	4.51	0.268	0.9303
Hearts (g)	2.18	2.08	2.25	2.18	2.22	0.091	0.7462
Gizzards (g)	4.37	4.14	4.40	4.25	4.25	0.206	0.8998
Small intestines (cm)	55.61	50.57	50.59	52.22	52.33	1.345	0.0836
[2]CCW (g)	164.6	167.6	176.5	168.8	171.1	6.99	0.8039
[3]WCW (g)	168.8	169.7	176.9	169.3	172.9	7.16	0.9025
Dressing %	69.57	67.92	69.78	69.97	69.34	1.005	0.6263

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]WCW: warm carcass weight.
[3]CCW: cold carcass weight.
[4]SEM: standard error of the mean.

Table 5.8 shows that experimental diets had no significant effect on the meat quality parameters measured immediately after slaughter of female Japanese quails. Meat pH ranged from 6.07 to 6.24, meat temperature ranged from 27.38 to 28.25 °C, meat lightness (L^*) ranged from 48.59 to 50.86, meat redness (a^*) ranged from 2.75 to 3.37, meat yellowness (b^*) ranged from 12.65 to 13.45, and meat chroma ranged from 12.95 to 13.80 and hue angle ranged from 1.32 to 1.37.

Table 5.8. The effect of protease-treated canola-based diets on meat quality parameters of 10-week old Japanese quails immediately after slaughter

	[1]Diets						
	CON	CM0	CM10	CM20	CM30	[2]SEM	*P*-value
Meat pH	6.07	6.09	6.24	6.23	6.19	0.061	0.1846
Temperature (°C)	28.04	28.25	27.84	27.38	27.57	0.276	0.1987
*L**	50.86	49.37	48.80	48.61	48.59	0.850	0.3074
*a**	2.97	2.79	2.81	2.75	3.37	0.409	0.8147
*b**	13.45	12.96	13.31	12.65	13.08	0.603	0.8950
Chroma	13.80	13.28	13.61	12.95	13.54	0.651	0.9027
Hue angle	1.35	1.37	1.36	1.36	1.32	0.024	0.6717

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]SEM: standard error of the mean.

Table 5.9 indicates that for meat quality parameters measured 24 h post-slaughter, dietary treatments had no significant influence on the pH (6.24–6.35), temperature (9.69–11.27 °C), *L** (47.3–48.8), *a** (3.43–5.06), *b** (5.44–6.90), chroma (6.47–8.54) and hue angle (0.94–1.05) of Japanese quails.

Table 5.9. The effect of protease-treated canola-based diets on meat quality parameters of 10-week old Japanese quails 24 h post-slaughter

	[1]Diets						
	CON	CM0	CM10	CM20	CM30	[2]SEM	*P*-value
Meat pH	6.35	6.24	6.26	6.29	6.28	0.046	0.5080
Temperature (°C)	9.69	9.73	10.46	11.27	10.82	0.587	0.2703
*L**	48.8	47.3	48.2	48.1	47.6	0.538	0.400
*a**	4.05	5.06	4.09	3.43	3.84	0.451	0.170
*b**	6.60	6.86	6.90	5.44	6.52	0.435	0.150
Chroma	7.77	8.54	8.05	6.47	7.58	0.560	0.423
Hue angle	1.03	0.94	1.04	1.02	1.05	0.038	0.231

[1]Diets: CON = control diet (a commercial growers mash with no canola meal inclusion), CM0 = control diet in which 17.5% of soybean meal was replaced with canola meal, CM10 = CM0 diet treated with 10% of protease enzyme, CM20 = CM0 diet treated with 20% of protease enzyme and CM30 = CM0 diet treated with 30% of protease enzyme.
[2]SEM: standard error of the mean.

Figure 5.1 shows that dietary treatments had no influence ($P > 0.05$) on cooking losses and peak positive force. Cooking losses ranged from 21.24 to 23.07 %, while the peak positive force ranged from 2.21 to 3.51 N.

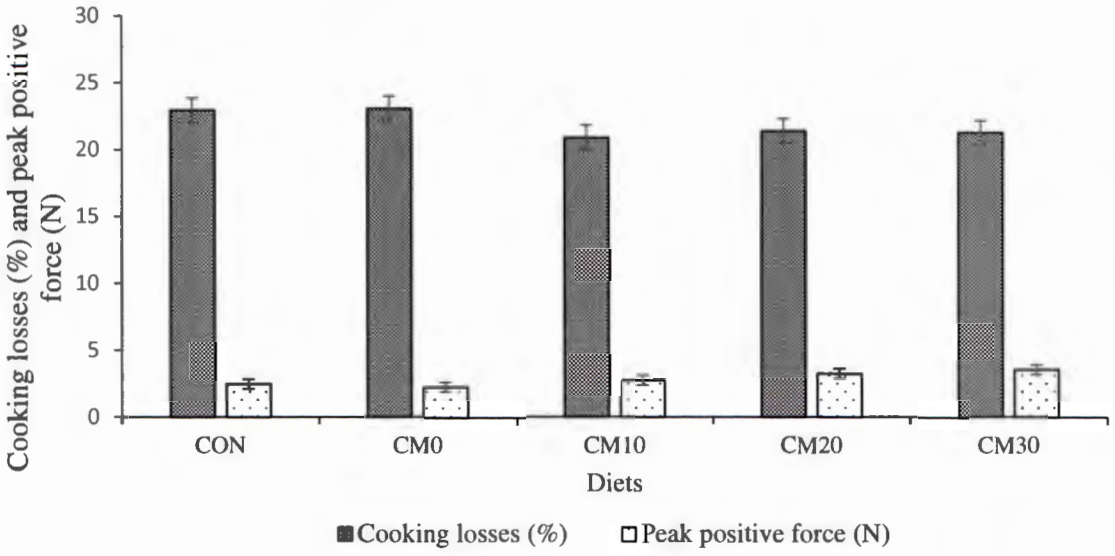

Figure 5.1. Cooking losses (%) and peak positive force (N) of Japanese quails as influenced by dietary treatments

5.4 Discussion

To the best of our knowledge no study has attempted to improve the performance of Japanese quails fed canola-based diets using exogenous protease enzyme. However there are numerous studies investigating the efficacy of this enzyme in pigs and broiler production (Romero *et al.*, 2013; Cowieson & Roos, 2016; Karimzadeh *et al.*, 2017). This study, therefore, represents the first attempt to use graded levels of exogenous protease to improve the feed value of CM in place of soybean. Repeated measures analysis showed no significant diet × week interaction effect on AWFI, AWG and GFR demonstrating that diets had no influence on the growth performance of the quails as they grew older.

Results from this study showed that inclusion of the protease mono-enzyme did not improve the utilisation of a CM-based quail diet as indicated by the no differences observed between the untreated control diets and the protease-treated CM-based diets in terms of feed intake, weight gain and GFR across weeks and for the entire duration of the study. These findings were in line with the conclusions of Marsman *et al.* (1997) who

observed no effect on weight gain and feed conversion ratio of chicks fed exogenous protease-treated soy-based diets. The explanation to these findings can be attributed to our initial findings reported in Chapter 3 that Japanese quails can only tolerate inclusion levels of up to 125 g/kg of canola-SBM diets. Indeed, Sariçiçek *et al.* (2005) found that addition of CM reduced feed consumption, which can be related to the presence of other ANF that cannot be acted on by the protease.

Although this study aimed at optimising the CM at 17.5% highest inclusion level in place of SBM using an exogenous protease as a strategy to reduce feed costs, still voluntary feed intake was reduced when compared to the FI values reported in Chapter 3. Another explanation for the no differences observed in terms of growth performance can be due to the use of the prescribed inclusion level (0.2 g/kg) of the protease that is meant for broilers. Even though three levels (0.1 g/kg, 0.2 g/kg and 0.3 g/kg) of protease were used to improve the utilisation, none prompted optimal quail performance suggesting that these inclusion rates may have been too low to trigger physiological changes in the quails.

Haematological and serum biochemical parameters were also not influenced by the inclusion of protease as they fell within the normal range reported by Ali *et al.* (2012). Considering the fact that several scholars reported improved feed utilisation, increased growth rates and desirable GFR values when exogenous proteases have been used (Simbaya *et al.*, 1996; Ghazi *et al.*, 2003; Freitas *et al.*, 2011; Cowieson & Roos, 2016; Stefanello *et al.*, 2016), none of these findings were reported for female Japanese quails. In fact the proteases used by these scholars were not fully described in terms of characteristics and activities. In addition, results from multi-enzymes are difficult to interpret because of the different enzymatic activities from the incorporated single enzymes. Discrepancies in the use of protease have been reported including adverse

responses to the enzyme, nutrient imbalances and failure of the enzyme to target its substrate (Cowieson *et al.*, 2006; Cowieson & Ross, 2016). Indeed, differences and inconsistencies relating to efficacy of various exogenous proteases have been revealed on a number of occasions. However, the differences are difficult to explain because of the limited details on the type of proteases applied (Kaczmarek *et al.*, 2014; Mahmood *et al.*, 2017).

Furthermore, size of internal organs and carcass characteristics were not altered by the inclusion of exogenous protease, emphasizing the protease enzyme's failure to trigger any measurable physiological changes. Dietary treatments also had no significant effect on meat colour, meat temperature, meat pH, meat cooking losses and peak positive force values of female Japanese quails, further indicating the ineffectiveness of protease with regards to meat quality traits.

5.5 Conclusions

Inconsistencies on reported results from the use of exogenous protease among scholars can be related to differences in terms of type and activity of the enzyme. The current study showed that protease-treated diets stimulated similar responses in terms of growth, haemo-biochemical status and meat quality traits as the canola-based diet. It was, therefore, concluded that the inclusion of exogenous protease enzyme does not improve the utilisation of a CM-based quail diet.

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6 CHAPTER SIX – GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

According to the United Nations the current human population is anticipated to grow to 9.8 billion in the year 2050 (United Nations, 2017). This expected growth adds more pressure on researchers and nutritionists to find more alternatives to feed the increasing human population. Generally, in the poultry industry, too much focus has been directed in improving and increasing the production of broilers to provide animal protein to consumers at lower costs. This is because white meat is generally considered cheaper and healthier than red meat (Muchenje *et al.*, 2009). Furthermore, chicken meat lacks religious restrictions (Jaturasitha, 2004). However, considering how fast the human population grows, there is a need to redirect attention to the production of other fast-growing avian species such as Japanese quails on a commercial scale.

Japanese quails are wild birds that have recently evolved in the poultry industry due to their desirable production characteristics such as rapid growth rates, early sexual maturity at 6 weeks of age, high laying intensity with short generation intervals, high resistance against avian diseases, meaning no vaccinations and other drug related treatments required (Randall & Bolla, 2008; Puspamitra *et al.*, 2014; Nasar *et al.*, 2016; Mnisi *et al.*, 2017). For this reason, quail farming on its own can be considered organic, which is preferred by many consumers. In addition, several matured quails can be reared in a floor space that can be used for one chicken. All these positive attributes have prompted attempts to improve and intensify their production.

Quails are considered game birds and have a recommended game-bird diet. However, these diets are difficult to access and they are very expensive and as a result resource-poor farmers are left with no option but to use chicken-based diets to feed the quails. In fact, most of the research conducted in quails used chicken-based diets as a way to reduce feed costs, which according to Ding *et al.* (2016), account for approximately 60 to 75% of production costs. When addressing the reason behind the increased feed costs, Beski *et al.* (2015) state that the use of expensive vegetable protein sources such as soybean during feed formulations is the reason behind increased animal feed prices.

As explained in Chapter 1, the reason behind the increased market prices of soybean is due to the fact that it is food for human beings and is also included in diets of pigs, dairy cows and all poultry species. The nutritive value of soybean is well-documented with high crude protein content and a well-balanced amino acid profile (Kocher *et al.*, 2002; Ravindran, 2013). As economists say, increased demand leads to increased prices, therefore continuous use of SBM during feed formulation is not cost-effective and can result in failure of the animal enterprise. Resource-poor farmers tend to use diets low in crude protein as a strategy to reduce feed costs and increased flexibility in feed formulation (Bercovici & Fuller, 1995). However, Aletor *et al.* (2000) demonstrated that when low CP diets are fed to birds, growth performance is compromised compared to birds fed high CP diets. This means that if soybean is to be substituted or replaced, the alternative protein source that will be used should have a high CP content with highly digestible essential amino acids and must not negatively affect the animal's health status. One such alternative is canola meal, a by-product acquired after oil extraction (Canola Council of Canada, 2009; Canola Council of Canada, 2015).

Canola meal has high CP content with an amino acid profile that can be fairly comparable to soybean (Wickramasuriya *et al.*, 2015). Canola meal is a relatively inexpensive protein source with the potential to be used as an alternative to SBM in quail diets. However, its effect on growth performance, physiology and meat quality in Japanese quails is largely unknown and requires investigation. The use of CM in poultry nutrition has been limited by the presence of ANF in the canola that depresses feed intake and stagnate growth performance (Summers *et al.*, 1988). Liver haemorrhage mortalities and abnormalities have also been reported when different canola cultivars with varying glucosinolate levels were fed at 25% inclusion levels (Campbell & Slominski, 1991). Waibel *et al.* (1992), also reported decreased growth rates and GFR when CM was added at 20% inclusion level in in growing turkeys.

Subsequently, more efforts have been directed towards the reduction of the ANF present in canola through genetic modifications, in fact, according to the Canola Council of Canada (2015), for the plant to be called "canola" the co-products must contain less than 2% erucic acids and 30 μmol/g of glucosinolates. Campbell and Slominski (1999), reported no liver haemorrhage mortalities at inclusion levels of 25%. Recent studies also revealed that inclusion level of up to 20–25% promote excellent performance both in broiler and turkey production (Naseem *et al.*, 2006; Georgeta, 2009). A current study by Saki *et al.* (2017), revealed that CM can be used up to 10% (in place of 30% SBM) in laying Japanese quail diets. Chapter 3, however, showed that dietary inclusion of canola beyond 125 g/kg reduces voluntary feed intake and can compromise the performance of female Japanese quails. In order, to increase the inclusion level of CM up to 175 g/kg beyond the established tolerance level of 12.5% (Chapter 3) as a way to reduce feed costs, the use of feed additives such as exogenous enzymes can be a strategy to improve the utilization of CM in quails allowing its inclusion at higher levels.

Several studies have attempted to use exogenous enzymes to increase phosphorus, carbohydrate and protein digestibility in CM (Kocher *et al.*, 2003; Sariçiçek *et al.*, 2005; Romero *et al.*, 2013; Cowieson & Roos, 2016). According to Ding *et al.* (2016), the use of exogenous enzymes such as phytase, carbohydrases and enzyme mixtures represent a cost-effective way to improve feed utilisation efficiency, nutrient bioavailability and ultimately desirable physiological responses. It is also important to note that the efficacy of enzymes does not depend only on the nature of dietary substrates but also on the production strain or microbial origin of the enzymes (Collins *et al.*, 2005). Mahmood *et al.* (2017) states that in order to meet the high demands of AA by high performing poultry birds protein supply must be increased or exogenous enzymes such protease must be added to supplement the endogenous proteolytic system to increase the digestion of dietary protein.

As an effort to improve the utilisation of CM at 17.5 % inclusion level in place of SBM (which was shown to reduce voluntary feed intake in Chapter 3), Chapter 4 and 5 revealed that using exogenous enzymes alone does not improve the utilisation of CM by quails, suggesting that many strategies are required if canola is to be included at higher levels. Results revealed that both the untreated-canola diets and the enzyme-treated diets did not alter the physiological state of the Japanese quails, which is a true reflection that canola can be a potential alternative to replace soybean, although precautions prior and during feeding should be put in place in order to ensure excellent quail performance, health status and desirable meat quality attributes.

6.2 Conclusions and Recommendations

As an attempt to establish a tolerance canola inclusion level of CM in place of soybean in diets of female Japanese quails, the initial study showed that CM can be a potential replacement of soybean in quails' diets up to 12.5% without negatively affecting growth

performance, blood parameters and meat quality characteristics. However, inclusion levels beyond 12.5% may not be appropriate given that the highest (175 g/kg) canola inclusion level reduced feed intake in quails. Subsequent studies were then conducted to use exogenous enzymes to improve the utilization of CM in quails to allow its inclusion at higher levels. The use of exogenous carbohydrases did not improve the utilisation of a CM based quail diet as results revealed that carbohydrase-treated diets promoted similar performance in terms of growth response, health status and meat quality traits as the untreated canola-based diet.

Even when exogenous protease was applied, the results showed that protease-treated diets promoted similar performance in terms of growth response, health status and meat quality traits as the canola-based diet, suggesting that the inclusion of exogenous protease enzyme does not improve the utilisation of CM-SBM diets fed to Japanese quails. Literature revealed a lot of differences and discrepancies on the use of enzymes. It is, therefore, recommend that multi-enzyme mixtures be evaluated as strategies to optimise the utilization of CM-based diets in quails at higher inclusion levels instead of mono-component and/or double-component enzymes. Further research is required to develop an effective enzyme preparation and to determine the synergistic effects and dose responses of protease and carbohydrase enzymes.

6.3 Future research

Results from the current study revealed that the inclusion of canola meal in Japanese quail based diets promoted similar performance, in terms of growth performance, haematology, serum biochemistry, carcass characteristics and meat quality traits, as the soybean meal-based positive control diet, however inclusion levels greater than 12.5% can compromise growth performance. Subsequent studies also indicated that improving the utilisation of

CM at an inclusion rate of 17.5% using feed enzymes was not beneficial. Therefore, future research can be designed to investigate the incorporation of feed multi-enzymes with heat treated canola meal to improve its utilisation by quails. The use of mixed gender quails from an early age (one week old) can be a possible research for the future. Another possible research aspect is to perform a digestibility trial with individual live weights, morbidity and mortality rate, carcass and breast yields, and proximate composition of breast meat.

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7 LIST OF APPENDICES

Appendix 7.1. Publication in Animal Nutrition

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Original Research Article

Growth performance, haematology, serum biochemistry and meat quality characteristics of Japanese quail (*Coturnix coturnix japonica*) fed canola meal-based diets

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Canola meal

ABSTRACT

The present study investigated the effect of partial replacement of soybean meal (*Glycine max*) with canola meal (CM) (*Brassica napus*) on the growth performance, haematology, serum biochemistry and meat quality characteristics of female Japanese quails in a 35-day feeding trial. One hundred and forty 6-week-old quails 158.28 ± 11.919 g were randomly allocated to 5 isonitrogenous and isoenergetic experimental diets: control diet (CM0; with no CM inclusion); CM0 with 2.5% (CM25), 5.0% (CM50), 12.5% (CM125) and 17.5% (CM175) soybean meal replaced with CM. Average weekly gain (AWG) and feed conversion efficiency (FCE) were determined. Haematology, serum biochemistry, carcass traits and meat quality parameters were determined at slaughter. Quails fed CM175 had the lowest ($P < 0.05$) feed intake whereas no differences were observed among the other 4 diets. No dietary effects on AWG, FCE and haematological parameters were observed. Serum biochemical parameters were not influenced by diets with the exception of alkaline phosphatase (ALP), where quails fed CM25 had higher ALP (161.0 U/L) than those fed CM0 (37.25 U/L). Quails fed CM25 had the highest chroma (7.39) while those fed CM125 had the lowest (3.58) at 24 h post-slaughter. Diets had no influence ($P > 0.05$) on cooking losses and peak positive force of quail meat. It was concluded that CM can replace soybean in quail diets up to 12.5% without compromising growth performance, health and quality of meat. Inclusion levels beyond 12.5% promoted poor voluntary feed intake and thus may require feed additives to enhance utilization.

1. Introduction

The poultry industry is one of the largest animal agriculture sectors in South Africa. It has evolved rapidly over the past 100 years from backyard household production to highly sophisticated commercial production units (Bolton, 2015). The industry continues to evolve with the addition of new bird species, such as the Japanese quail (*Coturnix coturnix japonica*), to complement the existing species. Although a relatively recent addition to the South African poultry industry, quail farming already contributes high-quality dietary protein for human consumption (Khosravi et al., 2016). Farming quails is economically viable and technically feasible because quails are quite resistant to various diseases, reach sexual maturity at 6 weeks of age and easily adapt to various rearing conditions (Randall and Bolla, 2008). However, the major challenge in the long-term sustainability of quail production remains the cost of dietary protein and the supply of essential amino acids (Wickramasuriya et al., 2015; Rezaeipour et al., 2016). Due to the nature of their digestive system, quails require dietary protein

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of very high quality, much similar to what humans require (Beski et al., 2015). Therefore, there is a direct competition between birds and humans for soybean. This often results in relatively higher prices of soybean on the world market creating artificial food shortages among the poorest societies around the world. According to Scanes et al. (2004), the efficacy of a protein feedstuff for poultry depends on its capacity to supply adequate amount of essential amino acids required by the bird. The quality of soybean meal as a protein source for poultry is unquestionable. However, alternative dietary protein sources are required to help to alleviate the challenge of high feed costs encountered in quail production. Canola meal (CM) is one such relatively inexpensive protein source (34% to 39% CP) that has potential to be used as an alternative to soybean in quail diets (Mushtaq et al., 2007; Beski et al., 2015). Unfortunately, canola contains antinutritional factors, such as glucosinolates, erucic acid, phytic acid, non-starch polysaccharides and phenolics, and also has high fibre content (Bell, 1993; Swick, 1999). These components are known to reduce amino acid digestibility and contribute to suboptimal growth performance of birds offered CM (Wickramasuriya et al., 2015). Furthermore, the lower energy value of CM, when compared to soybean meal, limits its utility in high-energy quail feeds. Nevertheless, genetic selection has successfully reduced the concentration of some undesirable components such as erucic acid and glucosinolates (Woyengo et al., 2014; Canola Council of Canada, 2015).

Encouraging diversification of the South African poultry industry through economical feeding of new entrants, such as the Japanese quail, will help to reduce dependency of the industry on soybean meal, address food and nutrition insecurity, and ensure profitability. Advanced research on the physiological and production parameters of Japanese quail fed CM under intensive management system is scanty (Faruque et al., 2013). This study, therefore, presents a nutritional evaluation of CM as an alternative protein source for Japanese quails. The overall objective of the study was to investigate the effect of graded levels of CM, as partial replacement for soybean meal, on growth performance, haematology, serum biochemistry and meat quality. The study was, therefore, designed to answer the following research question: does partial replacement of dietary soybean with CM affect growth performance, blood parameters and meat quality traits in female Japanese quails?

2. Material and methods

The experiment was approved by the Animal Research Ethics Committee, North-West University (AREC-MC), approved (NWU-00521-16-A9).

2.1. Description of the study site

The feeding trial for female Japanese quails was conducted at the Molelwane Research Farm (North-West University, South Africa) (25°40.459′ S, 26°10.563′ E), which is founded at an altitude of 1,226 m above sea level in the North-West province. The ambient temperatures at the study site range from 27 to 37 °C during summer and from 3 to 25 °C in winter. Annual rainfall ranges from 300 to 600 mm.

2.2. Feed ingredients

Soybean meal (SBM) and all the other feed ingredients, except for CM, were bought from Opti Feeds Pty Ltd., Lichtenburg, South Africa. The CM was purchased from Southern Oil Pty Ltd., Western Cape, South Africa. Both soybean and canola were solvent-extracted meals.

2.3. Diet formulation

Five diets were formulated by replacing SBM in a commercial grower diet with graded levels of CM using Format of Opti Feeds Pty Ltd., Lichtenburg, South Africa. The isonitrogenous and isoenergetic experimental diets were formulated by replacing the SBM component with CM as follows: CM0 = control diet with no CM inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with CM, CM50 = control diet in which 5% of soybean meal was replaced with CM, CM125 = control diet in which 12.5% of soybean meal was replaced with CM and CM175 = control diet in which 17.5% of soybean meal was replaced with CM, producing 5 dietary treatments as shown in Table 1.

2.4. Chemical analyses

The formulated diets (CM0, CM25, CM50, CM125 and CM175) were milled (Polymix PX-MFC 90 D) to pass through a 1 mm sieve for chemical analyses. Diets were analysed using the methods of AOAC International (AOAC, 2005). Analyses were conducted for laboratory dry matter (DM; AOAC method 930.15), organic matter (OM; AOAC method 924.05) and crude protein (CP; AOAC method

Table 1
Gross and chemical composition of diets on an as-fed basis (g/kg, unless otherwise stated).

Item	Diets				
	CM0	CM25	CM50	CM125	CM175
Canola oil cake	0	25	50	125	175
Yellow maize–fine	698.6	686.9	670.2	618.2	595.1
Prime gluten 60	18.0	13.0	10.3	20.0	24.3
Full fat soya meal	50.7	71.7	104	185	174
Soybean meal (local)	196.7	168	130.7	19.3	0
Limestone powder-fine	14.5	14.2	13.8	12.8	12.2
Mono calcium phosphate	7.2	7.0	6.8	6.1	5.6
NaCl (salt-fine)	3.2	3.2	3.3	3.2	3.2
Sodium bicarbonate	1.7	1.6	1.6	1.6	1.6
Choline powder	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.8	2.8	2.9	2.9
L-threonine	0.4	0.4	0.4	0.2	0.0
Methionine	1.9	1.8	1.8	1.4	1.8
Grower-phytase	1.7	1.7	1.7	1.7	1.7
Coxistac	0.5	0.5	0.5	0.5	0.5
Olaquindox	0.4	0.4	0.4	0.4	0.4
Chemical composition					
Dry matter	88.65	88.71	88.82	89.14	89.06
Organic matter	83.84	83.88	83.98	84.32	84.22
ME, MJ/kg	12.1	12.1	12.1	12.1	11.8
Crude protein	18.00	18.00	18.01	18.61	18.94
Crude fat	4.162	4.830	5.363	6.725	6.244
Crude fibre	2.315	2.589	2.892	3.726	4.176
Calcium	0.85	0.85	0.85	0.85	0.85
Phosphorus	0.497	0.506	0.515	0.542	0.563
Sodium	0.18	0.18	0.18	0.18	0.18
Chlorine	0.3	0.3	0.3	0.3	0.3
Potassium	0.763	0.763	0.764	0.740	0.733
Lysine	1.079	1.085	1.091	1.105	1.110
Methionine	0.478	0.476	0.475	0.460	0.520
Threonine	0.705	0.710	0.715	0.729	0.733
Tryptophan	0.187	0.189	0.192	0.197	0.201
Isoleucine	0.739	0.733	0.731	0.744	0.750
Arginine	1.102	1.101	1.102	1.101	1.100
Leucine	1.692	1.662	1.637	1.706	1.728
Valine	0.844	0.847	0.850	0.888	0.908

CM0 = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal; CM50 = control diet in which 5% of soybean meal was replaced with canola meal; CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal; CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal. ME = metabolizable energy.

984.13). Crude fibre was determined using the ANKOM^{2000} Fibre analyser (ANKOM Technology, New York). Amino acids were determined by hydrolysing samples with 6 mol/L HCl (containing phenol) for 24 h at 110 ± 2 °C in glass tubes sealed under vacuum as described by Ravindran et al. (2005). Mineral content (calcium, phosphorus, sodium, chlorine and potassium) was analysed following the guidelines provided by the Agri-Laboratory Association of Southern Africa (AgriLASA, 1998). Metabolizable energy (ME) content was predicted using the near infrared reflectance spectroscopy SpectraStar XL (Unity Scientific, Australia).

2.5. Experimental design

One hundred and forty 1-week-old female Japanese quails were acquired from A and J Services Farm, Palmietfontein, South Africa. The quails were reared using a starter-mash of Opti Feeds Pty Ltd., Lichtenburg and had access to fresh water with infrared lamps heating (until 3 weeks of age) to provide warmth because they are very sensitive to low temperature. At 5 weeks of age, the quails were randomly allocated to 20 replicate pens (experimental units), with each pen carrying 7 female birds. The 5 dietary treatments were randomly allocated to the pens (4 replicate pens per diet) and the quails were reared until they were 10 weeks of age. The quails were allowed to adapt to the pens and diets for a week before the experiment commenced.

2.6. Feeding and bird management

Dietary treatments and fresh water were provided *ad libitum* and average daily feed intake was measured during weeks 6 to 10. The initial live-weights of the quails were measured at the beginning of the experiment (the first day of week 6). Thereafter, average live-weight was measured weekly by weighing all the quails in each pen. The live-weights were used to calculate growth rates. Average weekly feed intake (AWFI) was calculated as the difference between the feed offered and the refusals. Each quail was weighed weekly and the average weekly gain (AWG) was calculated as follows:

$$AWG\ (t_0,\ T) = \frac{W(T) - W(t_0)}{T - t_0},$$

where t_0 = initial time (day); T = final time (day); $W(T)$ = final body weight (g) and $W(t_0)$ = initial body weight (g). Feed conversion efficiency was calculated as weight gained divided by the amount of feed consumed.

2.7. Slaughter procedures, blood collection and analyses

At 10 weeks of age, all female Japanese quails were deprived of feed for a period of 13 h to guarantee the emptiness of the crop as guided by Ari et al. (2013). All quails were taken to Rooigrond chicken abattoir (Mafikeng, South Africa) for slaughter. According to Berg and Raj (2015), all the quails were gas stunned by exposing them to relatively low concentrations of carbon dioxide (<40% by volume in air), and then, once they were unconscious, exposed to a higher concentration (approximately 80% to 90% by volume in air). Thereafter, quails were live-hanged onto a movable metal rack that holds them upside down by their feet. Quails were then slaughtered by cutting the jugular vein with a sharp knife, and they were left hanging until bleeding ended. At the same time, about 4 mL of blood was collected from 2 quails randomly selected from each pen into 2 sets of sterilised bottles (purple-top tubes with anti-coagulant for haematology and red-top tubes without anticoagulant for serum biochemical analysis). Haematological parameters (erythrocytes, haemoglobin, haematocrit, mean corpuscular volume [MCV] and mean corpuscular haemoglobin [MCH]) were determined using an automated IDEXX LaserCyte Haematology (IDEXX Laboratories, Inc.). Mean corpuscular haemoglobin concentration (MCHC) was calculated as the ratio of MCH to MCV. Albumin, alkaline phosphatase, alanine transaminase, amylase, blood calcium, serum cholesterol, creatinine, globulin, blood glucose, lipase, blood phosphorus, total bilirubin, total protein and urea were analysed using an automated IDEXX Vet Test Chemistry Analyser (IDEXX Laboratories, Inc.).

2.8. Internal organs and carcass traits

Upon completion of bleeding, the quails were put in a defeathering machine. Afterwards, carcasses were immediately taken to the Animal Science Laboratory (North-West University, South Africa) for carcass measurements and meat quality parameters. The weights of the livers, clean gizzards, hearts, wings, thighs and the lengths of the small intestines were determined. Hot carcass weight (HCW) was measured before the carcasses were chilled for 24 h to acquire the cold carcass weight. The dressing out percentage was determined as the proportion of HCW to the slaughter weight.

2.9. Meat quality measurements

2.9.1. Meat pH and temperature measurements

Meat pH and temperature were recorded immediately after 24 h post-slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA, USA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) according to Stanford et al. (2003). After every 20 measurements, the pH meter was calibrated with pH 4 and pH 7 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland) at a temperature of 2 °C.

2.9.2. Meat colour

Colour of the meat (L* = lightness, a* = redness and b* = yellowness) was determined, immediately after slaughter and 24 h after slaughter, using a Minolta colour-guide (BYK-Gardener GmbH, Geretsried, Germany) on a 20 mm diameter measurement area and illuminate D65 light at 10° observation angle. The colour meter was calibrated using the green standard before measurements. Colour recording was done on the surface of the thigh muscle, which was allowed to bloom for 1 h on a polystyrene tray at 4 °C. Hue angle was calculated as $\tan(\theta) = \frac{a^*}{b^*}$, and chroma was calculated as $\sqrt{a^{*2} + b^{*2}}$ as guided by Priolo et al. (2002).

2.9.3. Cooking losses and meat tenderness

After weighing, breast samples were placed in an oven set at 130 °C for 20 min for determination of cooking losses. The following formula was employed:

$$\text{Cooking losses (\%)} = \frac{(\text{Initial weight} - \text{Final weight})}{\text{Initial weight}} \times 100.$$

After determination of cooking losses, breast samples were sheared perpendicular to the fibre direction using a Meullenet–Owens razor shear blade mounted on an Universal Instron apparatus (cross head speed = 200 mm/min, one shear in the centre of each core). The reported value represented the average positive peak force (N) measurements of each sample.

2.10. Statistical analysis

Weekly feed intake, AWG and feed conversion efficiency (FCE) data were analysed using the repeated measures procedure of SAS (2010). Overall feed intake, weight gain, feed conversion efficiency, blood parameters, carcass characteristics and meat quality data were analysed using the general linear model procedure of SAS (2010). The linear statistical model employed was as follows:

$$Y_{ik} = \mu + D_i + E_{ik},$$

where Y_{ik} = dependant variable, μ = population mean, D_i = effect of diets, and E_{ik} = random error associated with observation ik, assumed to be normally and independently distributed. For all statistical tests, significance was declared at $P < 0.05$. Least squares means was compared using the probability of difference option in the LSMEANS statement of SAS.

3. Results

Table 1 shows that the DM and OM content of experimental diets increased as canola levels increased. All dietary treatments were isoenergetic and isonitrogenous. Higher inclusion of canola resulted in higher crude fibre and crude fat content in the diets. Phosphorus levels were also shown to increase with graded levels of CM.

Repeated measures analysis showed no significant ($P > 0.05$) week × diet interaction effect on AWFI, AWG and FCE. Diets significantly affected AWFI in weeks 7 and 8, but not in weeks 9 and 10 (Table 2) with diet CM175 promoting the least AWFI in weeks 7 and 8. Diet CM50 promoted the highest AWFI in weeks 7 and 8. Table 2 shows that there were no significant differences in weight gain and FCE across all weeks.

There was a significant dietary effect on overall feed intake. Table 3 shows that Quails fed CM50 had higher overall feed intake than those offered CM175. The quails fed CM50 had the same ($P > 0.05$) overall feed intake as those fed CM0, CM25 and CM125. However, quails fed CM175 did not significantly differ with those fed diets CM0, CM25 and CM125 in terms of overall feed intake. There was no dietary effect ($P > 0.05$) on overall weight gain and FCE for the entire duration of the study.

For haematological parameters, diet had no significant effect on erythrocytes, haemoglobin, haematocrit, MCV, MCH and MCHC of Japanese quails.

With the exception of alkaline phosphatase (ALP), all biochemical parameters were not ($P > 0.05$) influenced by experimental diets (Table 4). Quails fed CM25 had higher ALP compared to those fed CM0. The quails fed diet CM0 had the same ($P > 0.05$) ALP levels as those fed CM50, CM125 and CM175. The birds fed CM25 did not significantly differ with those fed diets CM50, CM125 and CM175 in terms of ALP content.

Table 5 shows that there were no significant dietary effects on the size of internal organs of quails with the exception of length of small intestines. Quails fed CM50 had the longest small intestines, which did not differ ($P > 0.05$) with those fed CM0. However, the size of small intestines in quails fed CM0 was similar ($P > 0.05$) to those fed CM25, CM125 and CM175. Carcass characteristics and dressing out percentages were not influenced by diets ($P > 0.05$).

For meat quality parameters measured immediately after slaughter, experimental diets had no significant effect on meat pH, temperature, L^*, a^*, b^*, chroma and hue angle of Japanese quails. However, Table 6 shows that meat quality traits measured 24 h post slaughter with the exclusion of meat pH and chroma were not affected ($P > 0.05$) by dietary treatments. Quails fed CM25 had the same ($P > 0.05$) meat pH as those fed CM0 and CM175. Quails fedCM50 and CM125 had the same ($P > 0.05$) meat pH as those offered CM0 and CM175. Meat from Quails fed diet CM25 had higher chroma than those fed diet CM125. However, quails fed CM25 did not differ ($P > 0.05$) from those fed CM0, CM50 and CM175 in terms of meat chroma.

Dietary treatments had no influence ($P > 0.05$) on cooking losses and peak positive force. Cooking losses ranged from 16. 63% to 21.07%. The peak positive force ranged from 4.69 to 5.62 N.

4. Discussion

Determination of growth performance, haematological and biochemical parameters of quails is essential in order to evaluate the effectiveness of diets in optimising bird performance without compromising their health. Including CM in poultry diets at higher levels may be necessary to ensure adequate digestible

Table 2
Weekly feed intake, weekly weight gain and weekly feed conversion efficiency (FCE) in Japanese quails fed graded levels of canola meal (CM).

Item	CM0	CM25	CM50	CM125	CM175	SEM	Significance
Feed intake, g							
Week 7	197.8^{ab}	198.3^{ab}	221.9^{b}	219.4^{b}	188.1^{a}	6.20	**
Week 8	198.3^{ab}	197.0^{ab}	215.6^{b}	212.7^{b}	176.4^{a}	8.30	*
Week 9	216.8	212.7	239.1	212.9	201.3	9.09	NS
Week 10	221.1	207.4	243.0	225.3	203.5	9.38	NS
Weight gain, g							
Week 7	39.68	41.31	46.66	40.25	41.06	4.608	NS
Week 8	18.89	19.37	19.07	21.79	13.50	3.092	NS
Week 9	9.00	6.58	2.85	8.62	6.17	1.862	NS
Week 10	7.93	4.80	5.91	1.32	5.60	2.609	NS
FCE							
Week 7	0.201	0.208	0.209	0.184	0.217	0.019	NS
Week 8	0.095	0.099	0.089	0.103	0.075	0.015	NS
Week 9	0.041	0.031	0.012	0.041	0.030	0.0072	NS
Week 10	0.036	0.026	0.025	0.005	0.027	0.012	NS

CM0 = control diet with no CM inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with CM, CM50 = control diet in which 5% of soybean meal was replaced with CM; CM125 = control diet in which 12.5% of soybean meal was replaced with CM; CM175 = control diet in which 17.5% of soybean meal was replaced with CM; SEM = standard error of the mean; * = ($P < 0.05$); ** = ($P < 0.01$); NS = not significant.
[a,b] Within a row, different superscripts denote significant differences ($P < 0.05$) between dietary treatments.

Table 3
Overall feed intake, weight gain and feed conversion efficiency (FCE) of 10-week-old Japanese quails fed graded levels of canola meal (CM).

Item	CM0	CM25	CM50	CM125	CM175	SEM	Significance
Feed intake, g	833.9^{ab}	815.3^{ab}	919.6^{b}	870.2^{ab}	769.3^{a}	27.35	*
Weight gain, g	73.25	72.05	74.49	69.82	66.33	5.249	NS
FCE	0.361	0.361	0.335	0.321	0.349	0.024	NS

CM0 = control diet with no CM inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with CM; CM50 = control diet in which 5% of soybean meal was replaced with CM; CM125 = control diet in which 12.5% of soybean meal was replaced with CM; CM175 = control diet in which 17.5% of soybean meal was replaced with CM; SEM = standard error of the mean; * = ($P < 0.05$); NS = not significant.
^{a,b} Within a row, different superscripts denote significant differences ($P < 0.05$) between dietary treatments.

Table 4
Effect of experimental diets on serum biochemical parameters of 10-week-old Japanese quails.

Biochemical parameters	CM0	CM25	CM50	CM125	CM175	SEM	Significance
ALB, g/L	22.00	22.43	18.13	20.0	23.91	1.84	NS
ALP, U/L	37.25^{a}	161.0^{b}	111.1^{ab}	112.9^{ab}	81.82^{ab}	23.48	**
ALT, U/L	41.13	50.29	44.5	37.43	40.82	11.22	NS
AMYL, U/L	588.4	658.4	479.7	557.1	594.0	125.3	NS
CA, mmol/L	2.45	2.98	2.69	2.50	2.41	0.206	NS
CHOL, mmol/L	4.92	3.93	3.55	3.1	3.34	0.691	NS
CREA, µmol/L	11.63	12.43	19.38	15.0	16.36	3.68	NS
GLOB, g/L	30.0	32.29	25.13	27.71	31.0	2.59	NS
GLU, mmol/L	3.85	2.05	3.19	1.50	3.23	1.10	NS
LIPA, U/L	234.9	266.0	255.3	310.3	296.5	43.0	NS
PHOS, mmol/L	4.77	5.20	5.13	5.20	5.15	0.182	NS
TBIL, µmol/L	53.4	59.0	24.3	33.4	65.8	12.82	NS
TP, g/L	52.0	54.71	43.25	47.71	54.91	4.32	NS
Urea, mmol/L	1.28	1.74	1.23	1.63	1.85	0.347	NS

CM0 = control diet with no canola meal inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with canola meal; CM50 = control diet in which 5% of soybean meal was replaced with canola meal; CM125 = control diet in which 12.5% of soybean meal was replaced with canola meal; CM175 = control diet in which 17.5% of soybean meal was replaced with canola meal; SEM = standard error of the mean; ** = ($P < 0.01$); NS = not significant; ALB = albumin; ALP = alkaline phosphatase; ALT = alanine transaminase; AMYL = amylase; CA = blood calcium; CHOL = serum cholesterol; CREA = creatinine; GLOB = globulin; GLU = blood glucose; LIPA = lipase; PHOS = blood phosphorus; TBIL = total bilirubin; TP = total protein.
^{a,b} Within a row, different superscripts denote significant differences ($P < 0.05$) between dietary treatments.

Table 5
Internal organs, carcass characteristics and dressing out percentage of 10-week-old Japanese quails fed graded levels of canola meal (CM).

Item	CM0	CM25	CM50	CM125	CM175	SEM	Significance
Gizzard, g	3.13	3.02	3.17	3.24	3.1	0.22	NS
Heart, g	1.56	1.81	1.56	1.8	1.6	0.16	NS
Liver, g	4.51	4.23	4.5	4.18	3.7	0.37	NS
Wing, cm	9.61	9.06	9.75	9.09	9.56	0.26	NS
Thigh, cm	3.91	3.8	3.85	3.73	3.7	0.14	NS
Small intestine, cm	17.4^{ab}	16.28^{a}	18.57^{b}	16.36^{a}	16.95^{a}	0.36	***
HCW, g	137	117	145.3	125.5	151	11.76	NS
CCW, g	134.6	116.1	143.7	123.3	147.4	11.39	NS
Dressing out, %	55.7	54.8	60.4	58.6	61.7	3.025	NS

CM0 = control diet with no CM inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with CM; CM50 = control diet in which 5% of soybean meal was replaced with CM; CM125 = control diet in which 12.5% of soybean meal was replaced with CM; CM175 = control diet in which 17.5% of soybean meal was replaced with CM; SEM = standard error of the mean; *** = ($P < 0.001$); NS = not significant; HCW = hot carcass weight; CCW = cold carcass weight.
^{a,b} Within a row, different superscripts denote significant differences ($P < 0.05$) between dietary treatments.

Table 6
Effect of graded levels of canola meal (CM) on meat quality parameters of 10-week-old Japanese quails 24 h post slaughter.

Item	CM0	CM25	CM50	CM125	CM175	SEM	Significance
pH	6.48^{ab}	6.56^{b}	6.38^{a}	6.39^{a}	6.43^{ab}	0.035	**
Temperature, °C	16.83	19.83	18.76	17.93	18.55	0.297	NS
L*	50.89	49.35	48.23	49.36	49.49	1.036	NS
b*	5.48	6.79	4.13	3.09	4.01	0.707	NS
a*	2.4	2.71	2.63	1.62	2.16	0.437	NS
Chroma	6.06^{ab}	7.39^{b}	5.12^{ab}	3.58^{a}	4.72^{ab}	0.835	*
Hue angle	1.1	1.2	1.02	1.04	0.97	0.09	NS

CM0 = control diet with no CM inclusion, CM25 = control diet in which 2.5% of soybean meal was replaced with CM; CM50 = control diet in which 5% of soybean meal was replaced with CM; CM125 = control diet in which 12.5% of soybean meal was replaced with CM; CM175 = control diet in which 17.5% of soybean meal was replaced with CM; SEM = standard error of the mean; * = ($P < 0.05$); ** = ($P < 0.1$); NS = not significant; L* = lightness; b* = yellowness; a* = redness.
^{a,b} Within a row, different superscripts denote significant differences ($P < 0.05$) between dietary treatments.

amino acids (Khosravi et al., 2016). However, in this study the highest inclusion level of canola (CM175) was seen to reduce AWFI, which could be a result of higher amounts of fibre levels and non-starch polysaccharides in the diet, and in line with the findings of Naseem et al. (2006). However, the negative effect on feed intake was not supported by the findings of Rojas et al. (1985) and Leeson et al. (1987), who reported that canola inclusion of 15% to 20% had no adverse effects on chickens. However, it is important to note the difference in bird species used in these studies because the digestive capacity of chickens and quails may differ when challenged with CM. Several studies have indicated that increased fibre levels reduce voluntary intake, particularly for simple non-ruminants, such as quails, which have limited ability to utilise fibrous diets (Sarıçiçek et al., 2005). Campbell and Slominski (1991) reported that ingestion of glucosinolates can lead to reduced feed intake and liver damage. However, it is important to note that there was no evidence of toxicity as measured by liver size and enzymes suggesting that any antinutritional factors present were not systemically harmful to the quails. Indeed, quails had similar liver weights and alanine transaminase across all experimental diets. This could be because the concentration of glucosinolates in canola has been reduced to very low levels through genetic selection (Campbell and Slominski, 1991). Phytic acid in canola is known to reduce calcium availability (Summers et al., 1988), and this could be the reason why Quails fed CM175 had generally low blood calcium levels. Quails had similar blood protein concentration; this is in line with reports that blood protein is not influenced by partial changes of protein in the diet (Bovera et al., 2007). Diets also had no significant impact on serum albumin; this is not surprising as there is a strong relationship between total protein and albumin (Omidi and Ansari nik, 2013). Bovera et al. (2007) reported that blood urea levels are elevated by increased dietary crude protein, which explains why diets had no significant effect on blood urea, and because the diets were isonitrogenous. High blood phosphorus in quails fed diets with canola can be explained by the inclusion of phytase enzyme, which breaks down phytate—a compound made up of more than 60% of phosphorus (Selle and Ravindran, 2007). The control diet was shown to generally promote high levels of blood cholesterol when compared to canola-based diets, suggesting that feeding canola to quails can reduce cholesterol levels in meat, which is a desirable outcome as far as consumers are concerned. Esonu et al. (2001) reported that haematological constituents demonstrate a physiological response of birds to internal and external environments such as type of feed and behavioural feeding patterns. However, in this study diets had no significant influence on haematological parameters, which fell within the normal range for quails. This suggests that inclusion of CM in place of soybean in quail diets does not negatively influence the physiological status of the birds. Also the noteworthy is the fact that serum creatinine levels were similar across experimental diets indicating that including canola in quail diets has no negative effect on kidney function. Meat quality parameters immediately after slaughter were not influenced by dietary treatments. However, diets were shown to influence the meat pH of 24 h post slaughter, suggesting that meat pH changes with storage time. The longest small intestines were observed in quails fed CM0 and CM50, which is surprising given that longer small intestines can be an adaptive mechanism to deal with increased amount of fibre, as longer small intestines were expected in quails fed higher levels of CM. Diets had no influence on peak positive force, suggesting that substituting SBM with CM does not negatively affect meat texture.

5. Conclusion

This study reveals that CM can be a potential replacement of soybean in Japanese quails' diets because growth performance, physiological status and meat quality parameters were not negatively affected when CM was included. However, precautions may need to be taken when high amounts of canola are used, given that the highest (175 g/kg) canola inclusion level was shown to reduce feed intake in quails. We suggest that the use of feed additives may improve the utilization of CM in quails allowing its inclusion at higher levels.

Acknowledgements

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Appendix 7.2. Ethics approval certificate of study

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ETHICS APPROVAL CERTIFICATE OF STUDY

Based on approval by **Animal Research Ethics Committee, Mafikeng Campus (AREC-MC)** on **03/01/2017** after being reviewed at the meeting held on **25/11/2016**, the North-West University Institutional Research Ethics Regulatory Committee (NWU-IRERC) hereby **approves** your study as indicated below. This implies that the NWU-IRERC grants its permission that provided the special conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Towards the optimisation of canola meal as a dietary protein source for Japanese quails, Coturnix coturnix japonica.

Promoter/Supervisor: **Prof V. Mlambo**
Student: **C MNISI**

Ethics number:

N	W	U	-	0	0	5	2	1	-	1	6	-	A	9
Institution				Study Number						Year			Status	

Application Type: PhD

Commencement date: 2017-01-03 **Expiry date**: 2019-12-31 **Category:** 1

Special conditions of the approval (if applicable):

- Any research at governmental or private institutions, permission must still be obtained from relevant authorities and provided to the AREC-MC. Ethics approval is required BEFORE approval can be obtained from these authorities.

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following:

- *The study leader (principle investigator) must report in the prescribed format to the NWU-IRERC via AREC-MC:*
  - *annually (or as otherwise requested) on the monitoring of the study, and upon completion of the study*
  - *without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.*
- *Annually a number of studies may be randomly selected for an external audit.*
- *The approval applies strictly to the proposal as stipulated in the application form. Would any changes to the proposal be deemed necessary during the course of the study, the study leader must apply for approval of these amendments at the AREC-MC, prior to implementation. Would there be deviated from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.*
- *The date of approval indicates the first date that the study may be started.*
- *In the interest of ethical responsibility the NWU-IRERC and AREC-MC retains the right to:*
  - *request access to any information or data at any time during the course or after completion of the study;*
  - *to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process.*
  - *withdraw or postpone approval if:*
    - *any unethical principles or practices of the study are revealed or suspected,*
    - *it becomes apparent that any relevant information was withheld from the AREC-MC or that information has been false or misrepresented,*
    - *the required amendments, annual (or otherwise stipulated) report and reporting of adverse events or incidents was not done in a timely manner and accurately,*
    - *new institutional rules, national legislation or international conventions deem it necessary.*
- *AREC-MC can be contacted for further information or any report templates via Ethics-AREC-MC@nwu.ac.za or 018 299 2197.*

The IRERC would like to remain at your service as scientist and researcher, and wishes you well with your study. Please do not hesitate to contact the IRERC or AREC-MC for any further enquiries or requests for assistance.

Yours sincerely

Prof LA Du Plessis

Digitally signed by Prof LA Du Plessis
Date: 2017.01.18 08:20:35 +02'00'

Prof Linda du Plessis

Appendix 7.3. Publication in South African Journal of Animal Science

Exogenous carbohydrases do not improve the physiological and meat quality parameters of female Japanese quail fed canola-based diets

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Abstract

In an internally controlled environment, a feeding trial using 210 six-week-old female Japanese quail (189.63 ± 11.891 g liveweight) was conducted to evaluate the effect of carbohydrase-treated (*endo*-1.4-beta-xylanase 5600 TXU/g and *endo*-1.4-beta-glucanase 2500 TGU/g) canola-based diets on growth performance, haemo-biochemical parameters, carcass characteristics, and meat quality traits. Five isocaloric and isonitrogenous experimental diets were formulated: the control diet (CON) (commercial growers diet with no canola meal (CM) included); the control diet in which 17.5% of soybean meal was replaced with CM (CM0); and the CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, 10% and 15% (CM50, CM100 and CM150, respectively). Diets and clean water were offered *ad libitum* during the four-week experimental period. Average weekly feed intake (AWFI) and average weekly weight gain (AWG) were used to calculate feed conversion efficiency (FCE). In week 7, no dietary influence was observed on AWFI. In week 8 and week 9, CON stimulated lower AWFI compared with diet CM100. Diets had no significant influence on AWG, FCE, and haemo-biochemical parameters of Japanese quail. Adding carbohydrases had no significant effect on internal organs, carcass and meat quality traits of quail. It was therefore concluded that inclusion of exogenous carbohydrases alone did not improve the utilization of a canola meal-based quail diet. However, there is a possibility that utilization of higher canola levels would be enhanced through multi-enzyme combinations.

Keywords: Blood parameters, carcass traits, exogenous enzymes, growth soybean meal
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Introduction

Coturnix coturnix japonica, commonly known as Japanese quail, is a strain of birds that are noted for their high growth rate, early sexual maturity and market age, short generation intervals and resistance to several avian diseases (Kabir, 2004; Deka & Borah, 2008; Dauda *et al.*, 2014; Mnisi *et al.*, 2017). In countries such as Japan, India, and France and in most parts of southern Africa, quail farming is gaining attention from farmers, entrepreneurs and researchers (Minvielle, 2004; Dauda *et al.*, 2014) because it is economically viable, and can be an alternative source of animal protein for human consumption (Puspamitra *et al.*, 2014). However, feed costs, which constitute about 80% of total cost of production, remain a major challenge in poultry farming owing to the use of expensive feed ingredients during feed formulation (Elagib *et al.*, 2013). Soybean and full-fat soybean products are commonly used to provide high-quality protein (Woyengo *et al.*, 2014; Beski *et al.*, 2015; Vagadia *et al.*, 2017) for quail. However, the demand for soybean on the world market is high owing to competition between human beings and animals for this dietary protein source (Mnisi & Mlambo, 2017). The resultant higher market prices of soybean suggest that it is prudent to search for less expensive alternatives to this ingredient in quail diets. A possible candidate is canola meal (CM), a by-product of oil extraction, with a protein content ranging from 36% to 39% (Wickramasuriya *et al.*, 2015). However, canola contains anti-nutritional factors that are known to restrict its utilization in the poultry industry. Secondary plant compounds such as glucosinolates, erucic acid, phytic acid, non-starch polysaccharides (NSP), and high fibre content limit the value of canola as a major protein source in quail feed formulation. Several scholars have reported that canola is a potential protein source, but inclusion levels greater than 250 g/kg can cause detrimental effects and reduce performance in birds (Naseem *et al.*,

2006; Ahmad *et al.*, 2007; Min *et al.*, 2011). A recent report by Mnisi & Mlambo (2017) indicated that CM could be used to replace 12.5% soybean in female Japanese quail diets without compromising growth performance, health, and quality of meat. However, in the same study, increasing the canola inclusion level to 17.5% of soybean resulted in a reduction in feed intake. The application of exogenous enzymes to complement endogenous digestive enzymes of quail may increase the utilization and digestibility of CM (Cowieson & Bedford, 2009). Carbohydrases hydrolyse cell wall polysaccharides in canola such as pentosans and β-glucans (NSPs), and therefore reduce their encapsulating effect, resulting in enhanced availability of carbohydrates, proteins and other nutrients (Campbell & Bedford, 1992; Khajali & Slominski, 2012). Additionally, the inclusion of carbohydrases in other poultry species has been reported to increase nutrient bioavailability and improve bird performance (Choct, 2006; Romero *et al.*, 2013). Other beneficial effects of carbohydrases include modulation of intestinal microflora (Fernández *et al.*, 2000), augmentation of digestive enzymes in growing animals (Gracia *et al.*, 2003), improved access of endogenous enzymes to cell contents (Cowieson, 2005), and reduction of endogenous amino acid losses (Gao *et al.*, 2008; Cowieson & Bedford, 2009). The effectiveness of exogenous carbohydrases in quail diets is largely unknown because most research is focused on broiler nutrition. This study was therefore designed to investigate the effects of treating canola-based diets with graded levels of a carbohydrase on growth performance, haemo-biochemical parameters, carcass and meat quality traits of Japanese quail. The authors hypothesized that adding a carbohydrase multi-enzyme (*endo*-1.4-beta-xylanase (> 1–< 3 %; 5600 TXU/g, EC no. 232-800-2) and *endo*-1.4-beta-glucanase (> 0.3–< 1 %; 2500 TGU/g, EC no. 232-734-4) mixture to canola-based diets would improve growth response, haemo-biochemical parameters, carcass characteristics, and meat quality traits of female Japanese quail.

Materials and methods

The procedures used to rear and slaughter quail were reviewed and approved by the Animal Research Ethics Committee, North-West University (AREC-MC) (approval no. NWU-00521-16-A9). For the duration of the study, all efforts were made to ensure that the rearing of the Japanese quail complied with the guidelines for the care and use of research animals (South African Bureau of Standards, 2008).

The feeding experiment was conducted at Molelwane Farm of North-West University (25°40.459' S, 26°10.563' E), South Africa. Ambient temperature ranges between 27 °C and 37 °C in summer and between -3 °C and 25 °C in winter months, respectively. Annual rainfall ranges between 300 and 600 mm. Canola meal was acquired from Southern Oil (Pty) Ltd (Western Cape, South Africa) while soybean meal (SBM) was supplied by Multi Agric (Edms) Bpk (North West, South Africa). According to the suppliers, both meals were solvent extracted. Carbohydrase enzyme mixture was received from BASF (Pty) Ltd (Gauteng, South Africa). This was a thermo-resistant NSP complex, containing *endo*-1.4-beta-xylanase (> 1–< 3 %; 5600 TXU/g, EC no. 232-800-2) and *endo*-1.4-beta-glucanase (> 0.3–< 1 %; 2500 TGU/g, EC no. 232-734-4).

Five isonitrogenous and isocaloric experimental diets (Table 1) were formulated using the Format® Software (Optifeeds (Pty) Ltd, Lichtenburg, South Africa): CON: control diet (commercial growers diet with no canola meal inclusion), CM0: control diet in which 17.5% of soybean meal was replaced with CM; CM50: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%; CM100: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10%; and CM150: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%. These inclusion levels were based on the recommended level suggested by the supplier (0.1 g/kg inclusion rate). However, this recommended application rate was for chickens. As a result, three inclusion levels were investigated for quail, namely one level below the recommended inclusion level and another above it.

These experimental diets were analysed according to AOAC (2005) for laboratory dry matter (DM) (AOAC method no. 930.15), organic matter (OM) (AOAC method no. 924.05). The total nitrogen content was determined by the standard macro-Kjeldahl method (N) (AOAC method no. 984.13) and was converted to crude protein by multiplying by a factor of 6.25. Amino acids were determined by hydrolysing the diet samples with 6 M HCl (containing phenol) for 24 hours at 110 ± 2 °C in glass tubes sealed under vacuum as described by Ravindran *et al.* (2005). Crude fibre (CF) was determined using the ANKOM^{2000} fibre analyser (ANKOM Technology, New York) according to Van Soest *et al.* (1991). Mineral matter was analysed according to Agri Laboratory Association of Southern Africa guidelines (AgriLASA, 1998). Metabolizable energy (ME) content was predicted using near infrared reflectance spectroscopy SpectraStar XL (Unity Scientific, Australia).

Table 1 Gross composition (g/kg) of canola meal-based diets treated with a carbohydrase multi-enzyme

	Diets[1]				
	CON	**CM0**	**CM50**	**CM100**	**CM150**
Carbohydrases	0	0	0.05	0.1	0.15
Canola oilcake	0	175	175	175	175
Yellow maize – fine	698.6	595.1	595.1	595.1	595.1
Prime gluten 60	18.0	24.3	24.3	24.3	24.3
Full-fat soya meal	50.7	174.0	174.0	174.0	174.0
Soybean meal	196.7	0.0	0.0	0.0	0.0
Limestone powder	14.5	12.2	12.2	12.2	12.2
Mono calcium phosphate	7.2	5.6	5.6	5.6	5.6
Salt – fine	3.2	3.2	3.2	3.2	3.2
Sodium bicarbonate	1.7	1.6	1.6	1.6	1.6
Choline powder	0.8	0.8	0.8	0.8	0.8
Lysine	2.8	2.9	2.9	2.9	2.9
L-Threonine	0.4	0.0	0.0	0.0	0.0
Methionine	1.9	1.8	1.8	1.8	1.8
Grower – phytase	1.7	1.7	1.7	1.7	1.7
Coxistac	0.5	0.5	0.5	0.5	0.5
Olaquindox	0.4	0.4	0.4	0.4	0.4

[1]Diets: CON: control diet (commercial growers diet with no canola meal inclusion), CM0: control diet in which 17.5% of soybean meal was replaced with CM, CM50: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10%; and CM150: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%

Three-week-old Japanese quail were purchased from a farm called Quail Breeders, Gauteng, South Africa. The quail were initially reared using a commercial grower-mash diet purchased from Optifeeds (Pty) Ltd (Lichtenburg, North West, South Africa) and had access to fresh water at all times. Quails were reared until five weeks old to allow differentiation of gender. At five weeks old, 210 female quail were selected and then randomly allocated to 30 replicate pens (experimental units), with each pen having seven quail. These pens were in a form of standing cages with four partitions. The sizes of the pens were 100 cm length × 60 cm width × 30 cm height. The five experimental diets were randomly allocated to the pens (6 replicate pens per diet) and the quail were reared until they were nine weeks old. The quail were allowed to adapt to the pens and diets for a week before the experiment commenced.

Experimental diets and fresh water were provided *ad libitum* during the three-week experimental period. AWFI was calculated as the difference between the feed offered and the refusals collected the following morning before feeding. Quails were weighed weekly and AWG was calculated as follows:

$$ADWG\ (t_o, T) = \frac{W(T) - W(t_o)}{T - t_o}$$

Where: t_0 = initial time (days)
T = final time
$W(T)$ = final bodyweight (g)
$W(t_0)$ = initial bodyweight (g)

Weekly feed conversion efficiency was calculated as weight gained divided by feed consumed.

At nine weeks old, quail stopped growing and were taken to Rooigrond Poultry Abattoir (North West, South Africa) for slaughter. At the abattoir, all the quail were live-hung upside down by their feet on a rail and electrically stunned. Quails were then slaughtered by cutting the jugular vein with a sharp knife and left

hanging until bleeding stopped. After defeathering, quail were taken to the Animal Science Laboratory of North-West University to measure carcass characteristics, internal organs, and meat quality parameters.

At slaughter, about 4 mL of blood was collected from two quail randomly selected from each pen in two sets of sterilised tubes, one containing ethylene diamine tetra acetic acid as an anti-coagulant for haematology and the other without an anticoagulant for serum biochemical analysis. For haematology the tubes were stored in a cooler box with ice packs and for serum biochemistry, samples were stored at room temperature for a maximum of 45 minutes to clot and then refrigerated at 4 °C (Washington & Van Hoosier, 2012). All analyses were conducted within 48 hours of collection (Buetow *et al.*, 1999). Haematological parameters (erythrocytes, haemoglobin, haematocrit, leucocytes, lymphocytes, neutrophils and monocytes) were determined using an automated IDEXX LaserCyte Haematology Analyser (IDEXX Laboratories, Inc.). For serum biochemical analyses, amylase, glucose, lipase and triglycerides were analysed with an automated IDEXX vet test chemistry analyser (IDEXX Laboratories, Inc.).

The weights of the liver, cleaned gizzard, heart and length of small intestines were determined in the animal science laboratory. Hot carcass weight (HCW) was recorded immediately after slaughter. After chilling for 24 hours, the carcasses were re-weighed to obtain the cold carcass weight (CCW). The dressing out percentage was determined as the proportion of HCW to slaughter weight.

Meat pH and temperature were recorded immediately after slaughter and also 24 hours post slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) according to Stanford *et al.* (2003). After every 20 measurements, the pH meter was calibrated with pH 4 and pH 7 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland) at a temperature of 2 °C.

Colour of the meat (*L**: lightness, *a**: redness, and *b*:* yellowness) was determined using a Minolta colour-guide (BYK-Gardener GmbH, Geretsried, Germany), with a 20-mm diameter measurement area and illuminant D65-day light 10° observation angle. The colour meter was calibrated using the green standard before measurements were taken. Colour recording was done in triplicate on the surface of a freshly cut slice of the breast muscle allowed to bloom for 1 hour on a polystyrene tray at 4 °C. Hue angle was calculated as $\tan(\theta) = \frac{a^*}{b^*}$, and chroma was calculated as $\sqrt{a^{*2} + b^{*2}}$ as guided by Priolo *et al.* (2002).

After weighing, breast samples were placed in an oven set at 130 °C for 20 min to determine cooking losses. This formula was employed:

$$Cooking\ losses\ (\%) = \frac{initial\ \ weight - final\ \ weight}{initial\ \ weight} \times 100$$

After cooking, cylindrical samples (12.5 mm core diameter) of breast muscle was cored parallel to the grain of the meat, and sheared perpendicular to the fibre direction using a Warner Bratzler shear device mounted on a Universal Instron apparatus (crosshead speed 200 mm/minute, one shear in the centre of each core). The reported value represented the average peak force measurements of each sample in newtons.

All reported parameters were tested for normality using the NORMAL option in Proc Univariate statement before being subjected to analysis of variance. Weekly feed intake, weight gain and feed conversion efficiency data were analysed using repeated measure analysis (SAS, 2010). This statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk}$$

Where: Y_{ijk} = dependent variable
μ = population mean
D_i = effect of diets
W_j = effect of week
$(D \times W)_{ij}$ = effect of interaction between diets and week
E_{ijk} = random error associated with observation *ijk*, assumed to be normally and independently distributed

Blood parameters, carcass characteristics and meat quality data were analysed using the GLM procedure of SAS version 9.4 (SAS, 2010). The linear statistical model was:

$$Y_{ik} = \mu + D_i + E_{ij}$$

Where: Y_{ij} = dependent variable
μ = population mean
D_i = effect of diets
E_{ik} = random error associated with observation *ij*, assumed to be normally and independently distributed

For all statistical tests, significance was declared at $P < 0.05$. Least squares means (LSMEANS) were compared using the probability of difference option in the LSMEANS statement of SAS.

Results

All experimental diets were isocaloric and isonitrogenous, as indicated in Table 2. Inclusion of CM resulted in higher crude fibre and crude fat content of the diet. Canola-based diets tended to have higher phosphorus and methionine levels than the control diet, which did not include canola.

Table 2 Chemical composition (g/kg, unless otherwise stated) of canola meal-based diets treated with a carbohydrase multi-enzyme

	[1]Diets				
	CON	**CM0**	**CM50**	**CM100**	**CM150**
Proximate analysis					
Dry matter	88.65	89.06	89.06	89.06	89.06
[2]ME (MJ/kg)	12.10	11.80	11.80	11.80	11.80
Crude protein	18.00	18.94	18.94	18.94	18.94
Crude fat	4.16	6.24	6.24	6.24	6.24
Crude fibre	2.32	4.18	4.18	4.18	4.18
Mineral matter					
Calcium	0.850	0.850	0.850	0.850	0.850
Phosphorus	0.497	0.563	0.563	0.563	0.563
Sodium	0.180	0.180	0.180	0.180	0.180
Chlorine	0.300	0.300	0.300	0.300	0.300
Potassium	0.763	0.733	0.733	0.733	0.733
Amino acid profile					
Lysine	1.079	1.110	1.110	1.110	1.110
Methionine	0.478	0.520	0.520	0.520	0.520
Threonine	0.705	0.733	0.733	0.733	0.733
Tryptophan	0.187	0.201	0.201	0.201	0.201
Isoleucine	0.739	0.750	0.750	0.750	0.750
Arginine	1.102	1.100	1.100	1.100	1.100
Leucine	1.692	1.728	1.728	1.728	1.728
Valine	0.844	0.908	0.908	0.908	0.908

[1]Diets: CON: control diet (commercial growers diet with no canola meal included), CM0: control diet in which 17.5% of soybean meal was replaced with CM; CM50: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%; CM100: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10%; and CM150: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%
[2]ME: metabolizable energy

Repeated measure analysis showed no significant (P >0.05) week × diet interaction effect on AWG and FCE. However, a significant interaction effect was observed for AWFI. Table 3 indicates that diets significantly affected AWFI in weeks 8 and 9 (P <0.05), but not in week 7 (P >0.05). The CON diet promoted lower AWFI in week 8 (186.8 g) and week 9 (193.4 g) compared with CM100 in week 8 (222.8 g) and week 9 (233.8 g). In weeks 8 and 9, CON did not differ (P >0.05) from CM0, CM50, and CM150 in terms of AWFI. Quail on CM100 had similar (P >0.05) AWFI to CM0, CM50, and CM150. Table 3 shows that there were no significant differences in weight gain and FCE for the duration of the feeding trial.

Table 3 Weekly feed intake (g), weekly weight gain (g) and weekly feed conversion efficiency in Japanese quail fed graded levels of carbohydrase-treated canola-based diets

	[1]Diets					
	CON	**CM0**	**CM50**	**CM100**	**CM150**	**[2]SEM**
Feed intake						
Week 7	211.6	225.5	222.8	230.2	226.7	5.436
Week 8	186.8[a]	213.0[ab]	201.4[ab]	222.8[b]	198.5[ab]	7.925
Week 9	193.4[a]	217.1[ab]	207.0[ab]	233.8[b]	204.2[ab]	7.715
Weight gain						
Week 7	34.16	37.29	37.59	37.36	37.43	3.117
Week 8	6.82	9.22	9.91	7.26	5.43	2.124
Week 9	8.25	6.06	4.26	4.89	6.54	1.472
Feed conversion efficiency						
Week 7	0.162	0.167	0.171	0.163	0.165	0.015
Week 8	0.037	0.043	0.049	0.033	0.028	0.010
Week 9	0.042	0.027	0.021	0.021	0.032	0.007

[1]Diets: CON: control diet (commercial growers diet with no canola meal); CM0: control diet in which 17.5% of soybean meal was replaced with CM; CM50: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%; CM100: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10%; and CM150: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%
[2]SEM: standard error of mean
[a,b] In a row, dietary treatment means with common superscripts do not differ (P <0.05)

Diets had no significant effect on the haematological parameters of female Japanese quail, namely erythrocytes (3.10–3.45 $\times 10^{12}$/L), haemoglobin (12.08–13.78 g/dL), haematocrit (0.51–0.57 L/L), leucocytes (34.72–50.73 $\times 10^{9}$/L), lymphocytes (27.65–43.23 $\times 10^{9}$/L), neutrophils (3.53–7.59 $\times 10^{9}$/L) and monocytes (1.38–2.61 $\times 10^{9}$/L)) and serum biochemical parameters, namely amylase (300.4–512.9 U/L), lipase (54.42–79.92 U/L), glucose (15.37–16.45 mmol/L) and triglycerides (0.86–1.63 mmol/L).

There were no significant dietary influences on internal organs, carcass characteristics and dressing out percentage of quail (P >0.05). The weights of hearts ranged from 1.98 to 2.29 g, while those of gizzards ranged from 3.93 to 4.40 g. Liver weights ranged from 3.95 to 4.65 g and the length of small intestines ranged from 51.2 to 54.4 cm. HCW ranged from 143.2 to 164.3 g, whereas CCW ranged from 141.3 to 163.3 g. Dressing-out percentage ranged from 59.3 to 69.5%.

Table 4 shows that experimental diets had no significant effects on all meat quality parameters measured immediately and 24 hours post-slaughter, that is, meat pH, temperature, lightness (L^*), redness (a^*), yellowness (b^*), chroma and hue angle of Japanese quail.

Table 4 Effects of carbohydrase-treated canola-based diets on meat quality parameters of Japanese quail immediately and 24 hours after slaughter

	[1]Diets					[2]SEM
	CON	CM0	CM50	CM100	CM150	
At slaughter						
Meat pH	5.82	5.75	5.94	5.95	5.86	0.073
Temperature (°C)	22.57	23.62	22.38	23.67	23.42	0.609
*L**	48.13	48.36	47.11	47.47	46.81	0.861
*a**	1.33	1.13	1.27	1.46	1.32	0.183
*b**	9.10	9.71	8.87	8.74	9.65	0.396
Chroma	9.20	9.79	8.82	8.87	9.7	0.403
Hue angle	1.3	1.46	1.42	1.41	1.44	0.018
24 h post-slaughter						
Meat pH	6.9	6.65	6.38	6.49	6.59	0.125
Temperature (°C)	10.61	11.32	10.37	10.51	10.52	0.319
*L**	47.27	47.77	47.5	47.23	46.61	0.513
*a**	3.74	2.47	3.34	3.81	3.47	0.348
*b**	11.87	11.36	11.73	11.63	11.68	0.302
Chroma	12.45	11.66	12.21	12.26	12.20	0.348
Hue angle	1.27	1.36	1.29	1.26	1.28	0.025

[1]Diets: CON: control diet (a commercial growers diet with no canola meal inclusion), CM0: control diet in which 17.5% of soybean meal was replaced with CM, CM50: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%, CM100: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10% and CM150: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%
[2]SEM: standard error of mean

Experimental diets had no influence (P >0.05) on cooking losses and peak positive force (Figure 1). Cooking losses ranged from 23.60 to 25.70 %, and peak positive force ranged from 5.15 to 6.80 N.

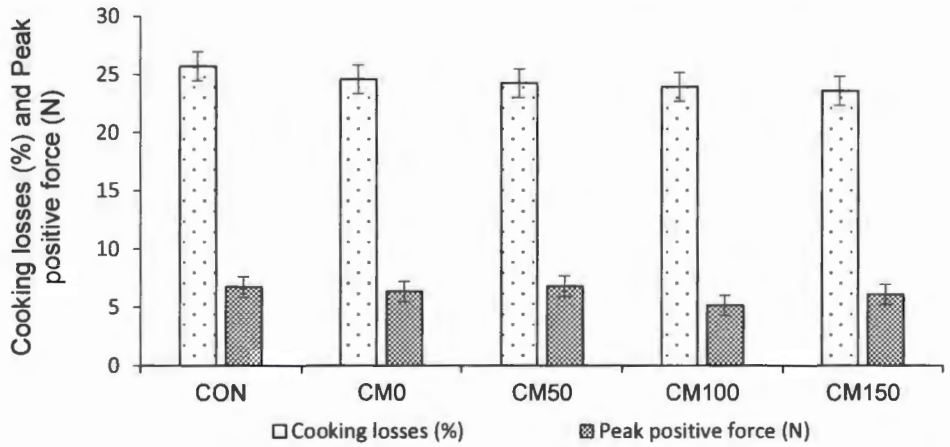

Figure 1 Cooking losses (%) and peak positive force (N) of Japanese quail as influenced by diets (CON: control diet (commercial growers diet with no canola meal included), CM0: control diet in which 17.5% of soybean meal was replaced with CM; CM50: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 5%; CM100: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 10%; and CM150: CM0 diet in which a carbohydrase multi-enzyme was added at a rate of 15%)

Discussion

According to Wickramasuriya *et al.* (2015), CM has higher methionine content than SBM and this explains why the inclusion of CM in quail diets in this study increased methionine levels, which is the major limiting amino acid in birds (Canola Council of Canada, 2009). Repeated measure analyses revealed a significant diet × week interaction effect on average weekly feed intake, signifying that the influence of diet on feed intake changed as the quail grew older. In week 8 and week 9, the control diet promoted lower AWFI compared with CM100, suggesting a compensatory feeding by the quail owing to dilution of nutrient concentration, which could have affected feed intake. Since the inclusion level of CM was 17.5% in place of soybean, it is likely that the NSP content in the canola would have induced a decrease in feed intake. However, the addition of the carbohydrases was intended to degrade the NSP and thus facilitate the absorbance of nutrients and improve weight gain. According to Walugembe *et al.* (2014), feeding fibrous diets in poultry tend to increase feed intake as a way of compensating for the reduced nutrient concentration in feed. However, according to Mnisi & Mlambo (2017), dietary inclusion of canola beyond 125 g/kg reduces feed intake and could compromise the performance of female Japanese quail.

The application of exogenous carbohydrase enzymes has been reported to improve feed utilization, nutrient digestibility and weight gain in birds fed fibrous diets (Gracia *et al.*, 2003; Adeola & Bedford, 2004; Romero *et al.*, 2013). Single enzymes such as xylanases and glucanases are mostly incorporated in poultry diets, representing more than 80% of the global carbohydrase market (Adeola & Cowieson, 2011). However, in this study, treating the canola-based diets did not improve feed intake, weight gain, or FCE, which agreed with the findings of Simbaya *et al.* (1996), Meng & Slominski (2005), and Mushtaq *et al.* (2007), who reported no effect of multi-carbohydrase enzyme in chickens fed CM-based diets. Additionally, Jia *et al.* (2012) and Radfar *et al.* (2017) found no improvement in broilers fed diets containing 150 g/kg of CM supplemented with carbohydrase enzyme. The differences in the mode of action and performance of the carbohydrases could be attributed to variations in application methods and the activities of the enzymes that were used in various studies (Yuan *et al.*, 2008).

Experimental diets had no significant impact on haematological and serum biochemical parameters of female Japanese quail, which fell within the normal ranges for quail (Ali *et al.*, 2012). This suggests that carbohydrase treatment of quail diets did not influence the physiological and pathophysiological status of the birds. It was expected that supplementation with carbohydrase would reduce the activity of pancreatic digestive enzymes, which would be manifested partly as lower serum amylase activity. Triglycerides values were also within the normal ranges for quail, indicating that there were no diet-induced modifications in the energy and fat metabolism of quail. Carbohydrase-treated diets did not affect the sizes of internal organs, carcass characteristics, pH, temperature, and colour of the meat, which were similar to those of quail fed an untreated CM-based diet. These findings are in agreement with Gracia *et al.* (2003), who observed no effect of carbohydrase single enzyme supplementation on relative weights of broiler organs. Longer small intestines in quail offered CM-based diets have been observed by Mnisi and Mlambo (2017), and could be the result of an adaptive mechanism to deal with the increased amounts of fibre for efficient digestion and absorption of nutrients. Experimental diets had no significant influence on cooking losses and peak positive force values of female Japanese quail, suggesting that application of carbohydrases did not improve meat quality traits.

Conclusions

The current study revealed that carbohydrase-treated CM-based diets promoted similar performances in terms of growth response, health status and meat quality traits to the untreated CM-based diet. It was therefore concluded that the dietary inclusion of an exogenous carbohydrase multi-enzyme did not improve the utilization of a CM-based quail diet. However, there is a possibility that utilization of higher canola levels can be enabled through other types of enzymes targeting or countering various antinutritional factors.

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Conflict of Interest Declaration

The authors declare that they have no conflict of interests.

Authors' Contributions

CMM and VM designed the study. CCM, KGP and TBM conducted the feeding trial and data collection. CMM and VM analysed the data statistically and wrote the paper.

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