EFFECT OF THREONINE SUPPLEMENTATION LEVEL ON PERFORMANCE OF MALE ROSS 308 BROILER CHICKENS FED A DIET CONTAINING CELLULASE

by

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DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture (Animal Production) is my independent work and research and that it has not previously been presented as a study at this university or elsewhere. I further declare that all materials contained herein have been duly acknowledged.

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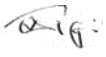
Prof Ngambi JW …  Date: 22/02/2024

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DEDICATION

This mini-dissertation is dedicated to my late father Albert Sebati and son Mfhangase Solomon Sebati, I hope they are proud of me.

Abstract

An experiment was conducted to evaluate threonine supplementation levels for optimal performance and carcass quality of male Ross 308 broiler chickens aged 22 to 42 days and fed diets mixed with a cellulase enzyme. The diets were isocaloric and isonitrogenous but with different dietary threonine levels. A complete randomized design was used. The treatments were CT_4 (4 g of threonine/kg DM), CT_5 (5 g of threonine/kg DM), CT_6 (6 g of threonine/kg DM) and CT_7 (7 g of threonine/kg DM) g of threonine/kg DM. A quadratic type of equation was used to determine dietary threonine levels for optimal performance of the chickens.

Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study had no effect (P>0.05) on feed intake, dry matter (DM) digestibility, live weight, live weight gain, feed conversion ratio (FCR), metabolizable energy (ME) intake and nitrogen retention of male Ross 308 broiler chickens. Dietary threonine levels had no (P>0.05) effect on gut organ digesta pH values, gut organ lengths and gut organ weights of Ross 308 broiler chickens aged 42 days. Dietary threonine level had no effect (P>0.05) on wing weights of the chickens aged 42 days. However, threonine level in the diet affected (P<0.05) carcass, thigh, drumstick, breast and abdominal fat weights of the chickens. Carcass, thigh, drumstick and abdominal fat weights were optimized at dietary threonine levels of 1.58, 3.12, 3.52 and 5.13 g of threonine /kg DM, respectively.

Dietary threonine level had no effect (P>0.05) on meat lightness and yellowness of the chickens. However, threonine level in the diet affected (P<0.05) red colour of the meat. A dietary threonine level of 5.80 g per kg DM was calculated to result in optimal redness of the meat. Threonine levels did not affect (P>0.05) thigh, drumstick, and breast pH values of the chickens. However, dietary threonine level affected (P<0.05) wing and abdominal fat pad pH values; and a dietary threonine level of 6.1 g/kg DM was calculated to result in optimal wing and abdominal fat pad pH values of male Ross 308 broiler chickens. Dietary threonine level did not affect (P>0.05) meat flavour and juiciness values. However, dietary threonine level affected (P<0.05) meat tenderness, shear force and water holding capacity values of the chickens. A dietary threonine level of 4.78 g per kg DM was calculated to result in optimal meat tenderness of the chickens.

The results obtained indicate that threonine levels required for optimal performance of the chickens are within the recommended dietary levels of 4 to 8 g/kg DM for broiler chickens, possibly indicating that diets containing cellulase require additional threonine to utilize additional energy generated.

Keywords: threonine, feed intake, body weight, FCR, meat colour, meat pH, digesta pH, digesta length, gut organ weight and sensory evaluation.

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CHAPTER 1

INTRODUCTION

1.1. Background

Livestock commodities are in high demand all over the world, and poultry meat is one of the most sought-after commodities. Feed is the foundation for profitable broiler production. On average, feed accounts for around 80-90% (NAFIS, 2017) of total cost of production. Poultry meat is the world's largest source of protein. Adequate nutrition is essential for chickens' health, fertility and performance. Higher growth rates mean that chickens need diets high in digestible energy and protein. Modern broiler diets are based on digestible amino acids to reduce costs and improve feed efficiency. The provision of diets high in protein devoid of any amino acid deficiency is especially important in the early nourishment of young chickens (Dibner, 2003). Poultry diets must have the right balance of essential nutrients to meet the chicken's nutritional requirements. The levels of threonine required for optimal productivity in Ross 308 broiler chickens are changing as a result of breed improvements (Kidd *et al*., 2004). There has been a high increase in chicken productivity due to efficient breeding, but a relatively low increase in chicken productivity as a result of improved nutrition (Talentire *et al*., 2016). Threonine is one of the major components of body protein. It plays an important precursor role as lysine and serine precursors (NRC, 1994). These amino acids play an important role in growth and tenderness of the meat (Oyadrin-Waldroup, 2002). Thus, data on dietary threonine levels for optimal performance of Ross 308 broiler chickens can be useful in devising strategies for improving productivity and carcass characteristics of these chickens.

1.2. Problem statement

Chickens are an important protein source in all countries (Mbajiorgu *et al*., 2011). Broiler chickens grow fast when given feeds containing required nutrients. However, chicken feeds contain 3-4% crude fibre which broiler chickens cannot digest because they don't produce cellulase enzymes that digest fibre. This is why cellulase enzymes are normally added to the chicken feeds to enhance fibre digestibility and hence chicken performance (NRC, 1994). Thus, addition of cellulase in the diet increases digestibility and hence availability of extra energy at the tissue level. The proper utilization of the additional energy at the tissue level requires that other nutrients, such as threonine, are also provided. Chickens cannot synthesize threonine and must be

provided in the feeds (Maynard *et al*., 2022; NRC, 1994). The information on required levels of threonine for optimal performance of broiler chickens fed diets containing cellulase is limited and not conclusive**.**

1.3 Scientific contribution of the study

This study will generate data on dietary threonine supplementation levels for optimal feed intake and digestibility, live weight gain, feed conversion ratio, meat quality and carcass characteristics of Ross 308 broiler chickens aged 22 to 42 days and fed a diet containing a cellulase enzyme. Information obtained from this study will assist in improving voluntary intake, growth rate, immune system responses, digestibility and meat quality of Ross 308 broiler chickens. Improvement in broiler chicken growth and meat quality will, hopefully, result in improved nutrition and income for Ross 308 broiler chicken farmers.

1.4. Rationale

A lot of research is being done to improve quality and quantity of broiler chicken meat, particularly breast meat (Waleed, 2017; Rezaei *et al*., 2004). Optimization of broiler chicken meat production requires that feeds contain nutrients in required amounts by the chickens. Threonine is an essential amino acid and it is a precursor of serine and lysine (Kidd, 2000; Kidd and Kerr, 1996). Lysine and serine promote growth in chickens (Ojano-Diranin *et al*., 2002). Threonine requirements for chickens vary from 5.9-8.0g/kg (Waleed, 2017; Kidd and Kerr, 1997; NRC, 1994). Chickens fed diets deficient in threonine have poor feed conversion ratio, growth and low meat yields (Maynard *et al*., 2022; Waleed, 2017; Kidd *et al*., 2004; Dozier *et al*., 2001; Kidd, 2000; Kidd *et al*., 1999; NRC, 1994). However, conclusive optimal threonine requirement data for broiler chickens fed diets having cellulase enzymes are currently limited. However, NRC (1994) suggested that chickens on diets having cellulase enzymes require higher dietary threonine levels than those on diets having no cellulase enzymes. It is, therefore, necessary to determine threonine levels for optimal performance of chickens fed diets having cellulase enzymes.

1.3. Aim

The aim of this study was to evaluate threonine supplementation levels for optimal performance and carcass quality of male Ross 308 broiler chickens aged 22 to 42 days and fed diets mixed with a cellulase enzyme.

1.4. Objectives

The objectives of this study were:

i. To determine the effect of threonine supplementation level in a diet containing a cellulase enzyme on performance of 22 to 42 days old male Ross 308 broiler chickens.
ii. To determine the effect of threonine supplementation level in a diet containing a cellulase enzyme on carcass quality of male Ross 308 broiler chickens aged 42 days.

1.5. Hypotheses

The hypotheses of this study were as follows:

i. Threonine supplementation level in a diet containing a cellulase enzyme does not affect performance of 22 to 42 days old male Ross 308 broiler chickens.
ii. Threonine supplementation level in a diet containing a cellulase enzyme does not affect carcass quality of male Ross 308 broiler chickens aged 42 days.

CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

According to Han *et al.*, (1992) threonine is the third limiting amino acid following methionine and lysine in broiler chicken's diets. Broiler cannot synthesis amino acid such threonine, hence threonine supplementation is required (Ayasan and Okan, 2006). Chickens' growth rate depends on the availability of amino acids that can synthesize proteins involved in many physiological processes (Geraert and Adisseo, 2010). The availability of amino acid is important in chicken rapid growth. Amino acids such as methionine, threonine and lysine are important for growth in broilers (Baylan *et al*. 2006). Is important to understand metabolic status of serine and glycine in relation to that of threonine (Barbour *et al*., 2008). Amino acids play many vital physiological roles, including thyroid function and lack of threonine in diet and other amino acids can results in diminished physiological function (Bender, 2012). Therefore, optimal dietary threonine requirements for chickens are vital. However, such information on Ross308 broiler chickens is not available. Thus, it is important to determine dietary threonine levels for performance of Ross308 broiler chickens.

2.2. Nutritional requirements of broiler chickens

Is important for chickens to consume all the required nutrient in order to be healthy and productive. Nutrition is an important part in poultry because the performances of chickens depend mostly on their nutrition. Each type of chickens its own specific nutritional requirements, and these requirements are always considered when formulating feed for chickens. Anything that included in feeds lower or higher would affect the performance of the chickens. Thus, it is important to know the nutritional requirements of broilers. Protein, fats and carbohydrates are important requirements for growth of the chickens. According to Mbajiorgu, (2010) chickens can change their feed intake over a considerable range of dietary energy levels in order to meet their daily energy requirements. Thus, dietary energy levels are used to set the levels of other nutrients including amino acids and proteins.

Balanced protein along with other nutrients is important in the growth of the broilers. The protein requirement of growing broilers includes the amount of protein needed for

maintenance plus the amount needed for tissue growth with an allowance for losses during digestion and metabolism. Improvement of energy level of the diet in the finishing stage and simultaneously slight decrease in protein level causes the broiler to consume more calories that it can use for growth. Total protein requirement can be met easily but in broiler chickens the required protein nutrition is amino acid, so it is important to satisfy amino acid requirement than satisfying crude protein requirement. All essential amino acids are important (NRC, 1994). According to Rezaei *et al*., (2004) Increasing content of threonine in the broiler diet results in increase in growth, carcass protein retention and a decrease in fat retention. However, knowledge about energy and threonine requirements of broiler chickens, such as the Ross308 broiler chickens, is limited and variable.

2.3. Use of cellulase enzymes in chicken diets

Cellulase enzymes are mostly used to supplement diets rich in non-starch Polysaccharides fed to broilers. The products of cellulase activity are more prone to fermentation by the microbial organisms that colonize the large intestine and cloaca of the gastrointestinal tract (Bedford and Apajalahti, 2001). Enzymes are used to catalyse the rate of a reaction but. Enzymes are involved in all catabolic and anabolic pathways of metabolism and digestion. Enzymes are important in poultry diets. Poultry do not produce enzymes for the Non-Starch Polysaccharide hydrolysis present in the cell wall of the grains and they remain unhydrolyzed. Enzymes break down the non-starch polysaccharides to reduce intestinal viscosity and improve the digestibility of nutrients by improving gut performance.

Enzymes have been shown to improve nutrient digestibility and performance when added to poultry diets (Saleh *et al*., 2003). The degree of improvement obtained by adding enzymes to the diet depends on many factors such as the dosage rate of enzymes, type of chicken, antinutritive factor in the diet, the physiology of the chicken, concentration of enzymes used, the age of the animal the chicken and type of gut micro flora present (Allen *et al.* 1995).

2.4 Biochemical functions of threonine

Threonine is an important amino acid with the molecular formula of C4H9NO3. Threonine is used in the biosynthesis of proteins. Threonine it involves an α-amino group, a carboxyl group, and a side chain containing a hydroxyl group (OH), whereby threonine become polar and uncharged amino acid. Threonine assists in the amalgamation of glycine and serine which aid the creation of elastin, collagen and muscle tissue. Additionally, threonine play an important role in the digestion, protein synthesis, and connective tissue formation (Piper *et al.*, 2017). Current studies support that the supplementation of threonine benefits in terms of energy metabolism. Threonine is acts as a nutritional modulator that affects the intestinal immune system via complex signaling networks, especially mitogen-activated protein kinase and the target of the rapamycin signal pathway. Threonine mainly serves as a substrate for protein synthesis, particularly mucin. In addition, threonine can enter the catabolic pathway, where it can be metabolized to a variety of important products such as glycine, acetyl Coa and pyruvate. Those products play an important role in host metabolism (Balch, 2000). The catabolism of threonine follows the glycine-independent or glycine-dependent pathway (House *et al*, 2001). Threonine not only serves as a precursor for protein synthesis but also as a signalling molecule that can regulate the protein synthesis pathway. Threonine maintains intestinal homeostasis by acting on intestinal morphology, barriers, micro-organisms and immune function.

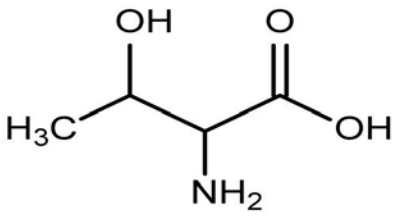

Figure 2. 01 The structure of threonine (Lehninger *et al*., 2000)

2.5 Effect of threonine on broiler chicken performance

According to Ojano and Waldroup (2002) adequate digestible threonine levels are needed to support optimum growth because it play an important role as a precursor of lysine and serine which are vital components of meat. The addition of threonine to broiler chicken diets increased productivity in terms of body weight, feed conversion ratio, breast weight and thigh weight (Estalkhzir *et al*., 2013). In addition, Rezaeipour *et al*., 2017 reported that threonine supplementation together with feed particle size improve feed conversion ratio, whereas threonine supplementation for the first 42 days after birth improved feed conversion ratio to levels above those seen with the addition of probiotics.

According to Al-Hayani (2017) broilers fed diet containing threonine enhanced carcass, breast and thigh weight. Kidd *et al*. (2003) indicated that broilers fed diets containing threonine have higher meat, liver and pancreas weight and lower thigh weight. Carcass weight improvement and quality characteristics are related to the role of Threonine on function of digestive enzymes and intestinal mucosa development. Growth performance is an important parameter to measure the effectiveness of feed given to broiler chickens. Environment is also influencing growth performance of broilers, depend on the dietary threonine levels (Kidd *et al.,* 2003).

2.6 Effect of threonine on broiler carcass characteristics

2.6.1 Effect of threonine on broiler chicken meat taste

Threonine is an essential amino acid that plays a crucial role in the growth and development of broiler chickens. While there is limited research specifically focusing on the direct effects of threonine on broiler chicken meat taste, its impact on meat quality attributes can indirectly influence taste perception. The improvement in carcass characteristics may be related to the function of threonine on digestive enzymes function and intestinal mucosa development (Rezaeipour and Gazani, 2014). Furthermore, the supplementation of threonine has been reported to increase the umami taste compounds such as glutamate and inosine monophosphate (IMP) in broiler meat (Liu *et al*., 2019). Umami taste is commonly associated with a savoury and pleasant flavour. Thus, the enrichment of these compounds through threonine

supplementation may enhance the overall taste experience of broiler chicken meat. It is important to note that while these studies suggest potential benefits of threonine supplementation on meat quality attributes, including taste, the specific taste perception by consumers can be subjective and influenced by various factors such as cooking methods, seasonings, and individual preferences. Further research is needed to comprehensively explore the direct effects of threonine on broiler chicken meat taste.

2.6.2 Effect of threonine on broiler chicken meat tenderness

Threonine supplementation in broiler diets has been shown to improve growth performance, carcass characteristics, and meat quality parameters. Studies have demonstrated that threonine supplementation can enhance the protein content and reduce the fat content in broiler chicken meat (Tang *et al.,* 2014; Zhao *et al.*, 2019). Increase in the amount of threonine level improves meat tenderness (Estalkhzir *et al.,* 2103). Temperature during slaughtering can also affect the toughness of the meat. Age of the chicken at slaughter also affects toughness of the meat. Tenderness decreases as an animal matures due to the cross-linking of collagen (Fletcher, 2002).

Higher marbling content carcasses also have more intermuscular and subcutaneous fat, which insulates the muscles during chilling and prevents the phenomena of cold shortening. As a result of a faster pH drop in fattier carcasses, which is linked to more tender meat, and slower cooling of fattier carcasses, which increases the activity of ageing enzymes, leading to more tenderness (Wood, 1997).

2.7 Conclusion

Optimization of broiler chicken meat production requires that feeds contain nutrients in required amounts by the chickens. Threonine is an essential amino acid, and it is a precursor of serine and lysine (Kidd and Kerr, 1996; Kidd, 2000). Lysine and serine promote growth in chickens (Ojano-Diranin *et al*., 2002). Threonine requirements for chickens vary from 5.9-8.0g/kg (Waleed, 2017; Kidd and Kerr, 1997; NRC, 1994). Chickens fed diets deficient in threonine have poor feed conversion ratio, growth, and low meat yields (Maynard *et al*., 2022; Waleed, 2017; Kidd *et al*., 2004). However, conclusive optimal threonine requirement data for broiler chickens fed diets having cellulase enzymes are currently limited.

CHAPTER 3

MATERIALS AND METHODS

3.1 Study site

This study was conducted at the University of Limpopo (UL) Livestock Unit, in Limpopo province, South Africa. The University of Limpopo lies at latitude 27.55^{o} S and longitude 24.77^{o} E. The mean ambient temperatures around the study area are 28^{o}C during winter and 36^{o}C in summer (Shiringani, 2007).

3.2 House preparation

The experimental house was cleaned properly with water and a disinfectant (Jeyes fluid). The house was left for 7 days after cleaning to break the life cycle of any disease-causing organisms that were not killed by the disinfectant. After proper drying, the house was divided into 16 floor pens. Fresh saw dust was placed on the floor to a level of 7cm. The heating of the house was done using 250 watt-infrared lights.

3.3 Acquisition of materials and chickens

A total number of 200 healthy day-old Ross 308 broiler chicks of both sexes were purchased from Angel feeds in Polokwane (Limpopo, South Africa). Yellow maize and wheat offals were purchased from NTK in Polokwane, whereas full-fat soya bean meal was acquired from Midfeeds in Nelspruit, Mpumalanga province. Fish meal and other micronutrients were purchased from Irvine's Africa in Pretoria, Gauteng province. The commercial diet, broiler mash (Starter ration) was acquired from Angel feeds in Polokwane. The equipment such as infrared lights, feeders, drinkers and disinfection medicines were acquired from NTK in Polokwane.

3.4 Experimental design, diets and procedures

A total of 200-day-old unsexed Ross 308 broiler chicks with an initial live weight of 40 $\pm$ 5g per chick were raised on a 22% crude protein commercial diet until they were 21 days old. Sixty-four male Ross 308 broiler chickens aged 22 days and weighing 580 ± 30g per bird were randomly assigned to 4 treatments (Table 3.01), with 4 replications, each having 5 chickens in a complete randomized design (SAS, 2008). During experimentation, the chickens were housed in one room divided into 16 pens

of equal size (9 m^2). The experimental housing was kept at ambient temperatures of 25 to 30℃. Diets were isonitrogenous and isocaloric and contained 200 units of cellulase per kg, but differed in threonine levels (Table 1). The threonine levels in the diets were at 4 (CT_4), 5 (CT_5), 6 (CT_6) or 7(CT_7) g per kg DM. The diets met nutrient requirements of broiler chickens (Table 3.2) as recommended by the National Research Council (NRC, 1994). The chickens were offered feed and water *ad libitum* and provided with light 23 hours per day.

Table 3.01 Diets

Code	Description*
CT_4	A diet containing cellulase and 4g of threonine/kg.
CT_5	A diet containing cellulase and 5g of threonine/kg.
CT_6	A diet containing cellulase and 6g of threonine/kg.
CT_7	A diet containing cellulase and 7g of threonine/kg.

* Amount of cellulase was 200 units per kg of feed as recommended.

3.5 Data collection

The initial live weight of each chicken was determined at the start of the experiment, thereafter, weekly weights were taken. The determined live weights were used to calculate body weight gain of the chickens. The daily feed intake was determined by subtracting the weight of feed leftover from the total weight of the feed that was given daily and the difference was divided by the total number of chickens in each replicate. The feed conversion ratio was calculated as the total amount of feed consumed divided by the weight gain of the chicken. Digestibility was done between the ages of 35 to 42 days. Digestibility was conducted in specially designed metabolic cages having separated watering and feeding troughs. Two birds were randomly selected from each replicate and used for digestibility determination. The chickens were given 3 days for adaptations in the metabolic cages. The excreta were collected from the fourth day until the seventh day at 8.00 hours every day. excreta were weighed, dried, and kept for nutrient analysis.

Table 3.02 Ingredients and nutrient composition of the diets

Feed ingredient	**Diet**			
	CT_4	**CT_5**	**CT_6**	**CT_7**
Maize (%)	51.00	51.00	51.00	51.00
Wheat offal (%)	12.00	12.00	12.00	12.00
Full fat soya (%)	28.00	28.00	28.00	28.00
Fish meal (%)	4.00	4.00	4.00	4.00
Limestone (%)	1.00	0.90	0.80	0.70
Bone meal (%)	2.95	2.95	2.95	2.95
Salt (%)	0.25	0.25	0.25	0.25
Vitamin Premix (%)	0.25	0.25	0.25	0.25
Dl-Methionine (%)	0.25	0.25	0.25	0.25
L-Lysine (%)	0.25	0.25	0.25	0.25
Coccidiostat (%)	0.05	0.05	0.05	0.05
Threonine (%)	0.0	0.1	0.2	0.3
Total (%)	**100**	**100**	**100**	**100**
Cellulase supplementation (units/kg)	200	200	200	200
Calculated analysis				
CP (%)	20.01	20.02	20.02	20.02
Energy (MJ/kg DM)	16.8	16.8	16.8	16.8
EE (%)	6.23	6.23	6.23	6.23
CF (%)	3.90	3.90	3.90	3.90
Ca (%)	1.40	1.40	1.40	1.40
Available P (%)	0.74	0.74	0.74	0.74
Lysine (%)	1.37	1.37	1.37	1.37
Methionine + Cysteine (%)	0.66	0.66	0.66	0.66
Threonine (%)	0.40	0.50	0.60	0.70

3.6 Gut organ and carcass characteristics, and sensory evaluation

At 42 days of age, all remaining chickens were sacrificed by manual cervical dislocation for gut organ and carcass evaluation. Each chicken was weighed and slaughtered according to rules and regulations of the University of Limpopo Animal Research and Ethics Committee. They were hanged upside down to completely bleed out, then defeathered, eviscerated, and finally weighed. The carcass weight of each chicken was measured, gut organ weight and digesta pH were measured using an electronic weighing scale and a pH meter (Crison, Basic 20 pH meter), respectively. The weight of carcasses, meat organs and gut organs were determined using an electronic weighing balance and expressed in grams.

The meat shear force assessment was done according to the method adapted by Dawson *et al.* (1991) using a texture analyser, Warmer-Bratzler shear force (WBSF) apparatus. Frozen samples of chicken breast meat were thawed for 24h at 2°C, tagged, and boiled on an electrical stove which was set at 35°C and finished at 70°C. Cooked meat samples were cooled down to room temperature (18°C) for at least 2 hours before WBSF measurements. Three cylindrical samples (12.5 mm core diameter) of each cut were cored parallel to the grain of the meat and sheared perpendicular to the fibre direction using a Warner-Bratzler shear force device mounted on a Universal Instron Apparatus (cross head speed = 200 mm/min, one shear in the centre of each core). The reported value in kg represents the average of three peak force measurements of each sample.

The method adopted by Pavelková *et al.* (2013) was used for meat sensory evaluation. Meat samples were frozen at -40°C for 3 days and then thawed for 7 hours at room temperature prior to cooking. The breast meat was prepared, and the skin was left on the meat samples, and nothing was added to the meat samples to enhance the flavour of the meat. The meat samples were covered with aluminium foil to prevent water loss and placed into an oven and cooked at 105°C for approximately an hour. After cooking, meat samples were cut into small 5 cm cubic pieces and served immediately. The meat was evaluated for tenderness, juiciness, and flavour. The sensory panel consisted of 20 trained panellists to rank each part on a 5-point ranking scale (Table 3.03). Each panellist was offered to drink lemon juice after tasting meat from each

treatment before proceeding to the next treatment as to wash out the previous treatment to avoid confusion of tastes.

Table 3.03 Evaluation score used by panellists

	Meat		
Score	**Flavour**	**Tenderness**	**Juiciness**
1	Very bad flavour	Too tough	Extremely dry
2	Poor flavour	Tough	Dry
3	Neither bad nor good flavour	Neither tough nor tender	Neither dry nor juicy
4	Good flavour	Tender	juicy
5	Very good flavour	Too tender	Too juicy

Source: Pavelková *et al.* (2013)

3.6 Chemical analysis

Dry matter contents of diet, excreta and meat samples were determined by oven-drying at 105°C for 24 hours. The ash contents of meat, excreta, diets and feed leftovers were determined by ashing the samples at 600℃ in a muffle furnace overnight. Feed, feed leftover and nitrogen retention were determined by Kjeldahl method (AOAC, 2010). Gross energy values of feeds, feed leftovers and excreta were determined using a bomb calorimeter at the University of Limpopo Animal Nutrition Laboratory guided by the method suggested by the Association of Analytical Chemists (AOAC, 2010). Ion-exchange chromatography (HPLC, University of Limpopo) was used to determine amino acids in feed, feed leftover and meat samples. Full analyses of feeds, feed leftovers and faeces were performed at the Pietermaritzburg laboratory, Kwa-Zulu Natal, South Africa according to methods by AOAC (2010).

3.7 Statistical analysis

Data on feed intake, digestibility, nitrogen retention, feed conversion ratio, growth rate, live weight, body weight gain, gut morphology, carcass characteristics and meat sensory attributes of Ross 308 broiler chickens aged 22 to 42 days were analysed by General Linear Model (GLM) using SAS version 9.3.1 software program, with threonine supplementation levels as the main effects. Where there were significant differences ($P<0.05$), the treatment means were separated using Least Significant Difference (LSD) test at 5% level of probability (SAS, 2008). The model $Y_{ij} = \mu + T_i + e_{ij}$ was applied where Yij = response variables in feed intake, digestibility, feed conversion ratio, body weight gain, live weight, body weight gain, gut morphology, carcass characteristics and meat sensory attributes of male Ross 308 broiler chickens; μ = constant; T_i = effect of threonine supplementation level; e_{ij} = random error.

The following quadratic equation (SAS, 2008) was used to determine threonine levels for optimal chicken performance:

$Y = a + b_1 x + b_2 x^2 + e$,

where Y = optimal performance (intake, digestibility, weight gain, etc.); a = intercept; b = coefficients; x = threonine level; $-b_1/2b_2$ = threonine level for optimal production; e = the error.

The relationships between threonine supplementation level in the diet and feed intake, digestibility, feed conversion ratio, body weight gain, live weight, gut organ weight, carcass characteristics and meat sensory attributes of male Ross 308 broiler chickens were modelled using the following linear regression equation (SAS, 2008): $Y = a + bx$ whereby Y = feed intake, digestibility, feed conversion ratio, growth rate, live weight, body weight gain, gut organ weight, meat pH, meat protein content, shear force, sensory attributes, etc. of male Ross 308 broiler chickens; a = intercept; b = coefficient of the linear equation; x = threonine supplementation level in the diet.

CHAPTER FOUR RESULTS

Results of the nutrient composition of the grower diets are presented in Table 4.01. The diets had similar (P>0.05) nutrient composition except that they had different (P<0.05) threonine levels.

Table 4.01 Nutrient composition of the diets

Nutrient	**Diet**			
	CT_4	**CT_5**	**CT_6**	**CT_7**
CP (%)	20.01	20.02	20.02	20.02
Energy (MJ/kg DM)	16.8	16.8	16.8	16.8
EE (%)	6.23	6.23	6.23	6.23
CF (%)	3.90	3.90	3.90	3.90
Ca (%)	1.40	1.40	1.40	1.40
Available P (%)	0.74	0.74	0.74	0.74
Lysine (%)	1.37	1.37	1.37	1.37
Methionine + Cysteine (%)	0.66	0.66	0.66	0.66
Threonine (%)	0.40	0.50	0.60	0.70

Results of the effects of threonine level on feed intake, live weight, live weight gain, feed conversion ratio (FCR), DM digestibility, feed intake and nitrogen (N) retention of male Ross 308 broiler chickens aged 22 to 42 days and fed diets containing a cellulase enzyme are presented in Table 4.02. Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study had no effect (P>0.05) on feed intake, live weight, live weight gain, feed conversion ratio (FCR), DM digestibility, feed intake and nitrogen (N) retention of male Ross 308 broiler chickens.

Table 4.02 Effect of dietary threonine level on growth performance (g/bird/day) and nutrient digestibility/retention (g/bird/day) of male Ross 308 broiler chickens aged 22 to 42 days.

	Treatment[#]			
Variable*	CT_4	CT_5	CT_6	CT_7
Feed intake (g/bird/day)	122 ± 28.68	116± 28.68	117± 28.68	150± 28.68
Live weight (g/bird at 42 days)	2592±51.7	2607±51.7	2626±51.7	2633±51.7
Live weight gain (g/bird/day)	95.1±1.32	95.8±1.32	96.6±1.32	97.0±1.32
FCR	1.28±0.21	1.21±0.21	1.21±0.21	1.50±0.21
DM digestibility (%)	64.0±6.12	67.9±6.12	69.6±6.12	72.8±6.12
ME intake (MJ/kg DM)	11.5±0.22	11.4±0.20	11.5±0.21	11.5±0.23
N-retention (g/bird/day)	29.6 ± 5.85	26.0 ± 8.25	40.9 ± 7.24	34.0 ± 4.90

* : Values presented as mean ± standard error (SE)

[#] : The treatments were diets containing cellulase and 4 (CT_4), 5 (CT_5), 6 (CT_6) or 7 (CT_7) g of threonine per kg DM

The effects of dietary threonine level on gut organ digesta pH values, lengths and weights of Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme are presented in Table 4.03. Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study had no effect ($P>0.05$) on gut organ digesta pH values, lengths and weights of chickens.

Table 4.03 Effect of dietary threonine level on gut organ digesta pH values, lengths and weights of Ross 308 broiler chickens aged 42 days

Variable*	Diet#				
	CT_4	CT_5	CT_6	CT_7	p-value
Gut digesta pH					
Large intestine	6.5±0.38	6.3±0.38	6.5±0.38	6.3±0.38	0.28
Small intestine	6.1±0.30	6.0±0.30	6.0±0.30	6.3±0.30	0.40
Caecum	6.4±0.27	6.5±0.27	6.4±0.27	6.3±0.27	0.46
Ileum	6.3±0.20	6.4±0210	6.3±0.20	6.2±0.20	0.59
Crop	6.2±0.22	6.0±0.22	6.1±0.22	6.2±0.22	0.73
Gizzard	6.1±0.13	6.1±0.13	5.9±0.13	6.1±0.13	0.54
Duodenum	6.5±0.32	6.1±0.32	6.4±0.32	6.2±0.32	0.10
Proventricular	6.5±0.32	6.3±0.32	6.4±0.32	6.1±0.32	0.05
Gut organ length (cm)					
Large intestines	13.6± 0.94	13.9±0.94	13.3±0.94	14.6±0.94	0.07
Small intestines	200.4±4.18	199.6±4.18	198.1±4.18	203.8±4.18	0.80
Caecum	22.3±0.89	22.3±0.89	23.5±0.89	23.6±0.89	0.64
GIT	233.2±8.06	231.5±8.06	230.1±8.06	236.8±8.06	0.57
Ileum	78.4±4.59	79.1±4.59	74.0±4.59	82.3±4.59	0.34
Duodenum	29.3±0.01	30.6±1.01	29.4±1.01	29.7±1.01	0.29
Jejunum	89.0±2.14	86.3±2.14	86.1±2.14	88.8±2.14	0.67
Gut organ weight (g)					
Large intestines	10.6±0.65	10.8±0.65	10.4±0.65	10.4±0.65	0.96
Small intestines	76.1±3.63	75.2±3.63	74.2±3.63	81.5±3.63	0.50
Crop	6.9±0.44	6.7±0.44	6.7±0.44	6.9±0.44	0.96
Proventricular	10.7±0.44	10.8±0.44	11.0±0.44	11.2±0.44	0.81
Gizzard	50.1±2.87	51.7±2.87	48.0±2.87	53.3±2.87	0.49
Caecum	18.6±1.20	17.5±1.20	19.1±1.20	19.5±1.20	0.66
GIT	282.7±9.48	270.2±9.48	265.9±9.48	273.6±9.48	0.64
Ileum	28.2±2.01	29.3±2.01	30.5±2.01	31.1±2.01	0.75
Liver	51.4±2.60	49.4±2.60	48.6±2.60	48.7±2.60	0.86
Pancreas	5.4±0.32	5.6±0.32	5.6±0.32	5.3±0.32	0.92
Duodenum	50.1±6.68	57.7±6.68	48.8±6.68	48.8±6.68	0.86

Heart	10.7±1.37	11.2±1.37	11.1±1.37	14.6±1.37	0.15
Jejunum	15.7±1.67	15.4±1.67	16.4±1.67	14.3±1.67	0.42
Spleen	34.9±2.22	34.2±2.22	33.3±2.22	35.6±2.22	0.89

* : Values presented as mean ± standard error (SE)

: The treatments were diets containing cellulase and 4 (CT_4), 5 (CT_5), 6 (CT_6) or 7 (CT_7) g of threonine per kg DM

Results of the effects of threonine level on carcass cut weights of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme are presented in Table 4.04. Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study had no effect (P>0.05) on wing weights of the chickens aged 42 days. However, threonine level in the diet affected (P<0.05) carcass, thigh, drumstick, breast and abdominal fat weights of the chickens. Male Ross 308 broiler chickens on a diet containing 4 g of threonine per kg DM had heavier (P<0.05) carcass weights than those on diets containing 5 or 7 g of threonine per kg DM. Similarly, chickens on a diet containing 5 g of threonine per kg DM had heavier (P<0.05) carcass weights than those on a diet containing 7 g of threonine per kg DM. However, chickens on diets containing 4 or 6 g of threonine per kg DM had similar (P>0.05) carcass weights. Similarly, chickens on diets containing 5 or 6 g of threonine per kg DM had the same (P>0.05) carcass weights. A dietary threonine level of 1.58 g per kg DM was calculated, with the use of quadratic equations, to result in optimal carcass weight of male Ross 308 broiler chicken meat ($Y = 2012.51 + 23.08x - 7.30x^2$, $r^2 = 0.660$).

Male Ross broiler chickens on diets containing 4 or 6 g of threonine per kg DM had heavier (P<0.05) thigh weights than those on a diet containing 7 g of threonine per kg DM. However, chickens on diets containing 4, 5 or 6 g of threonine per kg DM had similar (P>0.05) thigh weights. Similarly, chickens on diets containing 5 or 6 g of threonine per kg DM had the same (P>0.05) thigh weights. A dietary threonine level of 3.12 g per kg DM was calculated to result in optimal thigh weight of male Ross 308 broiler chicken meat ($Y = 296.46 + 13.68x - 2.20x^2$, $r^2 = 0.842$). Male Ross 308 broiler chickens on diets containing 4 or 6 g of threonine per kg DM had heavier (P<0.05) drumstick weights than those on a diet containing 7 g of threonine per kg DM. However, chickens on diets containing 4, 5 or 6 g of threonine per kg DM had similar (P>0.05) drumstick weights. Similarly, chickens on diets containing 5 or 7 g of

threonine per kg DM had the same (P>0.05) drumstick weights. A dietary threonine level of 3.52 g per kg DM was calculated to result in optimal drumstick weight of male Ross 308 broiler chicken meat ($Y = 221.97 + 10.56x + -1.50x^2$, $r^2 = 0.680$). Ross 308 broiler chickens on diets containing 5 or 6 g of threonine per kg DM had heavier (P<0.05) abdominal fat pad weights than those on a diet containing 7 g of threonine per kg DM. However, chickens on diets containing 4, 5 or 6 g of threonine per kg DM had similar (P>0.05) abdominal fat pad weights. A dietary threonine level of 5.13 g per kg DM was calculated to result in optimal abdominal fat pad weight of male Ross 308 broiler chicken meat ($Y = 38.52 + 23.09x + -2.25x^2$, $r^2 = 0.962$).

Table 4.04 Effect of dietary threonine level on carcass part weights of male Ross 308 broiler chickens aged 42 days

	Diet#				
Parameter*	**CT_4**	**CT_5**	**CT_6**	**CT_7**	**p-value**
Carcass (g)	2008.7^{a}±59.48	1883.4^{b}±59.48	1950.2^{ab}±59.48	1795.7^{c}±59.48	0.05
Thigh (g)	318.3^{a}±10.66	302.9^{ab} ±10.66	306.3^{a}±10.66	282.1^{b}±10.66	0.02
Drumstick (g)	242.3^{a}±8.02	231.0^{ab}±8.02	237.6^{a}±8.02	220.3^{b}±8.02	0.03
Wing (g)	204.3±5.80	196.2±5.80	195.2±5.80	193.9±5.80	0.58
Breast (g)	762.3^{a}±25.45	715.6^{ab}±25.45	752.4^{ab}±25.45	705.0^{b}±25.45	0.03
Abdominal fat (g)	18.1^{ab}±2.84	19.9^{a}±2.84	19.8^{a}±2.84	12.6^{b}±2.84	0.02

* : Values presented as mean ± standard error (SE)

a, b, c : Means with different superscripts in the same row indicate significant differences between treatments (P<0.05)

: The treatments were diets containing cellulase and 4 (CT_4), 5 (CT_5), 6 (CT_6) or 7 (CT_7) g of threonine per kg DM

Results of the effects of threonine level on meat colour of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme are presented in Table 4.05. Dietary threonine levels used in the present study had no effect (P>0.05) on meat lightness and yellowness of the chickens aged 42 days. However, threonine

level in the diet affected (P<0.05) red colour of the meat. Male Ross 308 broiler chickens on a diet containing 5 g of threonine per kg DM had meat with higher (P<0.05) red colour value than the meat from chickens on diets containing 4 g of threonine per kg DM. However, chickens on diets containing 5, 6 or 7 g of threonine per kg DM had meat with similar (P>0.05) red colour. Similarly, chickens on diets containing 4, 6 or 7 g of threonine per kg DM had meat with similar (P>0.05) red colour. A dietary threonine level of 5.80 g per kg DM was calculated to result in optimal redness of male Ross 308 broiler chicken meat ($Y = -15.85 + 8.405x - 0.725x^2$, $r^2 = 0.619$).

Table 4.05 Effect of dietary threonine level on meat colour of male Ross 308 broiler chickens aged 42 days

Variable*	**Diet#**				
	CT_4	**CT_5**	**CT_6**	**CT_7**	**p-value**
L*	-2.8±2.18	-2.8±2.18	-1.9±2.18	-2.7±2.18	0.56
a*	5.9^{b}±1.04	9.0^{a}±1.04	7.6^{ab}±1.04	7.8^{ab}±1.04	0.02
b*	-10.6±0.27	-11.0±0.27	-10.8±0.27	-10.9±0.27	0.40

* : Values presented as mean ± standard error (SE)

a, b : Means with different superscripts in the same row indicate significant differences between treatments (P<0.05)

: The treatments were diets containing cellulase and 4 (CT_4), 5 (CT_5), 6 (CT_6) or 7 (CT_7) g of threonine per kg DM

Hunter LAB test: L*: lightness; a*: redness and b*: yellowness

The effects of threonine level on meat pH values of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme are presented in Table 4.06. Dietary threonine levels did not affect (P>0.05) thigh, drumstick and breast pH values of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme. However, dietary threonine levels affected (P<0.05) wing and abdominal fat pad pH values of male Ross 308 broiler chickens aged 42 days. Male chickens on a diet containing 4 g of threonine per kg DM produced wings higher (P<0.05) pH values than those from chickens on diets containing 5, 6 or 7 g of threonine per kg DM. However, male broiler chickens on diets having 5, 6 or 7 g of threonine per kg DM produced wings with similar (P>0.05) pH values. A dietary threonine level of 6.1 g per kg DM was calculated to result in optimal wing pH value of male Ross 308 broiler

chicken meat (Y = 8.03 + -0.61x + 0.045x^2, r^2 = 0.933). The chickens on a diet containing 4 g of threonine per kg DM produced meat with higher (P<0.05) abdominal fat pad pH values than those from chickens on diets containing 5, 6 or 7 g of threonine per kg DM. However, chickens on diets having 5, 6 or 7 g of threonine per kg DM produced abdominal fat pads with similar (P>0.05) pH values. A dietary threonine level of 6.1 g per kg DM was calculated to result in optimal fat pad pH value of male Ross 308 broiler chicken meat (Y = -10.775 -1.525x + 0.125x^2, r^2 = 0.933).

Table 4.06 Effect of dietary threonine level on meat pH values of Ross 308 broiler chickens aged 42 days

Meat part*	**Diet#**				
	CT_4	**CT_5**	**CT_6**	**CT_7**	**p-value**
Thigh	6.4±0.04	6.4±0.04	6.4±0.04	6.4±0.04	0.13
Drumstick	6.4±0.04	6.5±0.06	6.4±0.04	6.4±0.06	0.23
Wing	6.4^a±0.03	6.2^b±0.03	6.2^b±0.03	6.2^b±0.03	0.0006
Breast	6.1±0.04	6.1±0.04	6.1±0.04	6.1±0.04	0.06
Abdominal fat	6.7^a±0.04	6.2^b±0.04	6.2^b±0.04	6.2^b±0.04	0.05

* : Values presented as mean ± standard error (SE)

a, b : Means with different superscripts in the same row indicate significant differences between treatments (P<0.05)

: The treatments were diets containing cellulase and 4 (CT_4), 5 (CT_5), 6 (CT_6) or 7 (CT_7) g of threonine per kg DM

Results of the effects of threonine level on meat sensory attributes of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme are presented in Table 4.07. Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study did not affect (P>0.05) meat flavour and juiciness values of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme. However, dietary threonine levels used in the present study affected (P<0.05) meat tenderness, shear force and water holding capacity values of male Ross 308 broiler chickens aged 42 days. Male Ross 308 broiler chickens on diets containing 4, 5 or 6 g of threonine per kg DM produced meat with higher (P<0.05) tenderness values than meat from chickens on diets having 7 g of threonine per kg DM. However, male broiler

chickens on diets having 4, 5 or 6 g of threonine per kg DM produced meat with similar (P>0.05) tenderness values. Male Ross 308 broiler chickens on diets containing 4 or 5 or 6 g of threonine per kg DM produced meat with higher (P<0.05) shear force values (harder) than meat from chickens on diets having 7 g of threonine per kg DM. However, male broiler chickens on diets having 4, 5 or 6 g of threonine per kg DM produced meat with similar (P>0.05) shear force values. Similarly, chickens on diets having 6 or 7 g of threonine per kg DM produced meat with the same (P>0.05) shear force values. Male Ross 308 broiler chickens on a diet containing 4 g of threonine per kg DM produced meat with higher (P<0.05) water holding capacity values than meat from chickens on diets containing 5, 6 or 7 g of threonine per kg DM. Similarly, chickens on diets containing 6 or 7 g of threonine per kg DM produced meat with higher (P<0.05) water holding capacity values than meat from chickens on a diet containing 5 g of threonine per kg DM. However, male broiler chickens on diets having 6 or 7 g of threonine per kg DM produced meat with similar (P>0.05) water holding capacity values. A dietary threonine level of 4.78 g per kg DM was calculated, with the use of quadratic equations, to result in optimal meat tenderness of male Ross 308 broiler chickens aged 42 days ($Y = -1.025 + 1.675x + -0.175x^2$, $r^2 = 0.972$).

Table 4.07 Effect of dietary threonine level on sensory evaluation of Ross 308 broiler chickens

Variable*	**Diet#**				
	CT_4	**CT_5**	**CT_6**	**CT_7**	**p-value**
Flavour	3.0±0.27	3.3±0.27	3.3±0.27	3.5±0.27	0.63
Tenderness	2.9^{a}±0.27	2.9^{a}±0.27	2.8^{a}±0.27	2.1^{b}±0.27	0.98
Juiciness	2.7±0.27	2.9±0.27	2.6±0.27	2.8±0.27	0.91
Shear force	10.8^{a}±0.66	10.8^{a}±0.66	9.9^{ab}±0.66	9.2^{b}±0.66	0.26
Water holding capacity	5.1^{a}±0.35	3.0^{d}±0.35	4.1^{b}±0.35	3.8^{c}±0.35	0.01

* : Values presented as mean ± standard error (SE)

a, b, c, d : Means with different superscripts in the same row indicate significant differences between treatments (P<0.05)

\# : The treatments were diets containing cellulase and 4 (CT_4), 5 (CT_5), 6 (CT_6) or 7 (CT_7) g of threonine per kg DM

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

The diets used in the present study contained 20% CP and 16.0 MJ of energy/kg DM. This met the recommended requirements for the broiler chickens aged 22 to 42 days (NRC, 1994). The diets contained similar levels of all the nutrients except threonine. The diets contained dietary threonine levels of 4, 5, 6 or 7 g per kg DM.

A lot of research is being done to improve quality and quantity of broiler chicken meat, particularly breast meat (Waleed, 2017; Rezaei *et al.,* 2004). Optimization of broiler chicken meat production requires that feeds contain nutrients in required amounts for the chickens. Threonine is an essential amino acid and it is a precursor of serine and lysine (Kidd, 2000; Kidd and Kerr, 1996). Lysine and serine promote growth in chickens (Ojano-Diranin *et al.,* 2002). Threonine requirements for chickens vary from 4-8 g/kg (Waleed, 2017; Kidd and Kerr, 1997; NRC, 1994). Chickens fed diets deficient in threonine have poor feed conversion ratio, growth and low meat yields (Maynard et *al.,* 2022; Waleed, 2017; Kidd *et al.,* 2004; Dozier *et al.,* 2001; Kidd, 2000; Kidd *et al.,* 1999; NRC, 1994). Conclusive optimal dietary threonine requirement levels for broiler chickens fed diets having cellulase enzymes are currently limited. However, NRC (1994) suggested that chickens on diets having cellulase enzymes require higher dietary threonine levels than those on diets having no cellulase enzymes. Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study had no significant effect on feed intake, DM digestibility, live weight, live weight gain, FCR, ME intake and nitrogen retention of male Ross 308 broiler chickens, possibly indicating that dietary threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study. These threonine levels are within the recommended dietary levels of 4 to 8 g/kg DM for optimal broiler chicken performance (NRC, 1994), possibly indicating that diets containing cellulase did not require additional threonine to utilize additional energy generated. Ospina-Rojas (2018), indicated that proper threonine supplementation in broiler diets can improve live weight and live weight gain of chickens. Bahadoran *et al*. (2015), observed that adding more dietary threonine to the diet improved growth rate, body weight gain and feed conversion ratio. Tugay *et al*. (2009) recommended a dietary threonine level of 7.5 g/kg DM for optimal feed intake in male broiler chickens aged 22 to 42 days. NRC (1994) suggested that a threonine level of 7.4 g/kg DM optimized carcass weight of 21 to 42 days old broiler chickens.

Dietary threonine levels of 4, 5, 6 or 7 g/kg DM had no effect on gut organ digesta pH values, lengths and weights of Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme, possibly indicating that dietary threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study. It seems addition of cellulase into the diets did not alter the threonine requirement levels for optimal gut organ digesta pH values, lengths and weights of Ross 308 broiler chickens.

Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study had no effect on wing weights of the chickens aged 42 days. However, threonine level in the diet affected carcass, thigh, drumstick, breast and abdominal fat weights of the chickens. Carcass, thigh, drumstick and abdominal fat weights were optimized at dietary threonine levels of 1.58, 3.12, 3.52 and 5.13 g of threonine per kg DM, respectively. These results are lower than those of Lemme (2001) which show that a dietary threonine level of 7.4 g/kg DM optimized carcass part weights of broiler chickens aged 22 to 42 days. Ciftci and Ceylan (2004) reported that an increase in dietary threonine increased breast meat yield of broiler chickens. NRC (1994) suggested that dietary threonine levels of 4 to 7 g/kg DM optimize meat yield of male Ross 308 broiler chickens aged 22 to 42 days. The results of the present study are lower than those observed by other authors (NRC, 1994; Lemme, 2001; Ciftci and Ceylan, 2004). It is suggested that cellulase inclusion in the diets might have improved the efficiency with which threonine is utilized for meat production. It is suggested that further studies be conducted to ascertain these findings.

Dietary threonine levels used in the present study had no effect on meat lightness and yellowness of the chickens aged 42 days, possibly indicating that threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study. However, threonine level in the diet affected red colour of the meat. A dietary threonine level of 5.80 g per kg DM was calculated, with the use of quadratic equations, to result in optimal redness of male Ross 308 broiler chicken meat. This is within the recommended levels for optimal red colour of chicken meat (Emans, 1994; NRC, 1994; Gous, 2014).

Results of this study indicate that threonine levels used in the present study did not affect thigh, drumstick and breast pH values of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme, possibly indicating that threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study. However, dietary threonine level affected wing and abdominal fat pad pH values of male Ross 308 broiler chickens aged 42 days. A dietary threonine level of 6.1 g per kg DM was calculated, with the use of quadratic equations, to result in optimal wing and abdominal fat pad pH values of male Ross 308 broiler chickens. El-Faham *et al*. (2017) observed that a higher threonine level of 8.7 g/kg DM improved drumstick pH values of broiler chickens aged 42 days. Abbasi *et al*. (2014) and Rezaeipour *et al*. (2012) recommended a lower dietary threonine level of 5.0 g/kg DM for optimal drumstick pH values of broiler chickens aged 22 to 42 days.

Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study did not significantly affect meat flavour and juiciness values of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme, possibly indicating that threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study. Amino acids play major roles in eliciting the characteristics of juiciness and flavour of foods (Kobayashi *et al*., 2009). Lawrie (2006) identified three compounds (free glutamic acid, 5'-inosinic acid and potassium ion) as the taste active components in chicken meat extracts. Glutamic and 5'-inosinic acid are favourites among consumers as they constitute a characteristic taste of chicken meat (Lawrie, 2006). Dietary threonine levels used in the present study affected meat tenderness, shear force and water holding capacity values of male Ross 308 broiler chickens aged 42 days. A dietary threonine level of 4.78 g per kg DM was calculated, with the use of quadratic equations, to result in optimal meat tenderness of male Ross 308 broiler chickens aged 42 days. Male Ross 308 broiler chickens on diets containing 4 or 5 or 6 g of threonine per kg DM produced meat with higher shear force values (harder) than meat from chickens on diets having 7 g of threonine per kg DM. Male Ross 308 broiler chickens on a diet containing 4 g of threonine per kg DM produced meat with higher water holding capacity values than meat from chickens on diets

containing 5, 6 or 7 g of threonine per kg DM. A study by Azzam *et al*. (2017) examined different dietary threonine levels and their effects on meat sensory attributes. They found that increasing threonine inclusion levels led to improved meat flavour attributes, resulting in more desirable sensory characteristics. Wang *et al.* (2019) investigated the effects of different dietary threonine levels on meat quality and flavour-related compounds in broilers. The authors observed that higher threonine inclusion levels positively influenced the concentration of certain flavour-related compounds in the meat, leading to enhanced flavour profiles. Smith *et al.* (2019) explored the effects of dietary threonine levels on meat shear force and water holding capacity in male Ross 308 broiler chickens. Their findings suggested that higher threonine inclusion levels in the diet influenced meat shear force and water holding capacity values in broiler chicken meat.

5.2 Conclusions

Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study had no effect on feed intake, DM digestibility, live weight, live weight gain, FCR, ME intake and nitrogen retention of male Ross 308 broiler chickens, possibly indicating that dietary threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study. These threonine levels are within the recommended dietary levels of 4 to 8 g/kg DM for optimal broiler chicken performance (NRC, 1994), possibly indicating that diets containing cellulase did not require additional threonine to utilize additional energy generated.

Dietary threonine levels used in the present study had no effect on gut organ digesta pH values, lengths and weights of Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme, possibly indicating that dietary threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study.

Dietary threonine levels used in the present study had no effect on wing weights of the chickens aged 42 days; however, threonine level in the diet affected carcass, thigh, drumstick, breast and abdominal fat weights of the chickens. Carcass, thigh, drumstick

and abdominal fat weights were optimized at different dietary threonine levels of 1.58, 3.12, 3.52 and 5.13 g of threonine per kg DM, respectively. These results indicate that dietary threonine requirements for carcass part weights of broiler chickens will depend on the production variable of interest.

Dietary threonine levels used in the present study had no effect on meat lightness and yellowness of the chickens. However, threonine level in the diet affected red colour of the meat and a dietary threonine level of 5.80 g per kg DM was calculated to result in optimal redness of male Ross 308 broiler chicken meat.

Results of this study indicate that threonine levels used did not affect thigh, drumstick and breast pH values of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme. However, dietary threonine level affected wing and abdominal fat pad pH values of the chickens; and a dietary threonine level of 6.1 g per kg DM was calculated to result in optimal wing and abdominal fat pad pH values of male Ross 308 broiler chickens. This dietary threonine level for optimal wing and abdominal fat pad pH values is within the recommended levels.

Dietary threonine levels of 4, 5, 6 or 7 g/kg DM used in the present study did not significantly affect meat flavour and juiciness values of male Ross 308 broiler chickens aged 42 days and fed diets containing a cellulase enzyme, possibly indicating that threonine levels required for optimization of these parameters were lower than or equal to the levels used in the present study. However, threonine levels used in the present study affected meat tenderness, shear force and water holding capacity values of male Ross 308 broiler chickens aged 42 days. A dietary threonine level of 4.78 g per kg DM was calculated, with the use of quadratic equations, to result in optimal meat tenderness of male Ross 308 broiler chickens aged 42 days.

5.3 Recommendations

Different dietary threonine levels optimized different production parameters. It is, thus, recommended that when formulating diets for male Ross broiler chickens, where cellulase enzymes are included in the diets, threonine levels should depend on the parameters of interest.

CHAPTER 6

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