EFFECT OF DIETARY THREONINE LEVEL ON PRODUCTIVITY AND CARCASS CHARACTERISCS OF ROSS 308 BROILER CHICKENS

NGOMANI DELISILE

2019

EFFECT OF DIETARY THREONINE LEVEL ON PRODUCTIVITY AND CARCASS CHARACTERISCS OF ROSS 308 BROILER CHICKENS

by

DELISILE NGOMANI

BSC AGRICULTURE (ANIMAL PRODUCTION)

A RESEARCH MINI-DISSERTATION

Submitted in fulfilment of the requirements for the degree MASTER OF SCIENCE IN AGRICULTURE (ANIMAL PRODUCTION)

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

Supervisor: Prof J. W. NG'AMBI

Co-supervisor: DR D BROWN

2019

DECLARATION

I declare that this mini-dissertation hereby submitted to the University of Limpopo for the degree of Master of Science in Agriculture (Animal Production) has not been submitted by me for a degree at this or any other university, this is my own work in design and execution, and that all materials contained herein has been duly acknowledged.

Signature…….. Date…….

Ms Delisile Ngomani

ACKNOWLEDGEMENTS

First and foremost, praises and thanks to the great God, the Almighty, for his showers of blessings throughout my study.

I would like to express my sincere gratitude to my supervisor, Prof J W Ng'ambi for his continuous support throughout this study. His expertise, invaluable guidance, affectionate attitude, patience and healthy criticisms added considerably to my experience. Without his continual inspiration, it would have not been possible to complete this study. I sincerely admire the contribution of my co-supervisor, Dr D Brown for his generous support, sympathetic attitude and unfailing help throughout this study. I appreciate the assistance given by Mr M.M. Ginindza during feed formulations and in the execution of data analysis.

I am grateful to my fellow MSc student, Naum Nyanese, for her patience, support, kind advice and interesting follow-up which gave me confidence and made the completion of this work possible. I have regarded our regular meetings as very enjoyable and beneficial to the quality of my work. Deep thanks are, also, given to my fellow MSc students, Grace Manyelo and Queen Paledi. I, also, wish to express my appreciation for the technical assistance provided by the University of Limpopo Animal Unit and Animal laboratory technician, Ms P.N. Nhleko. I would like to thank the National Research Foundation (NRF) and the Department of Agriculture, Forestry and Fisheries (DAFF) for sponsorship and financial support I received during the entire period of my study.

Special thanks go to my father, Sam Erick Ngomani, and my mother, Gladys Ivy Ngomani, who gave me courage and motivation to study further. I, also, wish to extend my special appreciations to my lovely sisters, Ophelia and Pretty, and my brothers, Dumisani and Ntoto, for their prayers, love, understanding, support and tolerance for the entire period of my study.

I feel a deep sense of gratitude to my late grandmother, Tsakani Mphephu Nkon'wani, who formed part of my vision and taught me the good things that really matter in life. I can only say proper thanks to her through my future work. The happy memory of my grandmother still provides persistent inspiration for my journey in this life.

DEDICATIONS

This dissertation is dedicated to God almighty for his guidance and courage throughout this study.

To my late grandfather, Richard Ngomani, may his soul continue to rest in peace.

ABSTRACT

Two experiments were conducted to determine the effect of dietary threonine level on production performance and carcass characteristics of Ross 308 broiler chickens. In each experiment the diets were isocaloric and isonitrogenous but with different dietary threonine levels.The first part of the study determined the effect of dietary threonine level on feed intake, growth rate, mortality and carcass characteristics of Ross 308 broiler chickens aged between Day 1-21. A total of 150 unsexed day-old chicks were used in a complete randomized design having 5 treatments (6.4, 7.5, 8, 8.5 and 9g of threonine/kg DM feed), replicated three times and having ten chickens per replicate. The second part of the study determined the effect of dietary threonine level on feed intake, digestibility, growth rate, mortality and carcass characteristics of male Ross 308 broiler chickens aged between Day 22-42. Seventy-five male chickens were used in a complete randomized design having 5 treatments (6.4, 7.5, 8, 8.5 and 9g of threonine/kg DM feed), replicated three times and having five chickens per replicate. A quadratic regression model was used to determine the optimal productivity of the chickens while a General Linear Model (GLM) procedures for the statistical analysis of variance was used to detect dietary treatment effects. Where there were significant differences ($P<0.05$), Turkey's honestly significant difference test (HSD) was used for mean separation.

The chickens were slaughtered at the ages of 21 and 42 days for Experiments 1 and 2, respectively, following ethical standards as recommended by the University of Limpopo Animal Research Ethics Committee (AREC/12/2017: PG). Two chickens per replicate for both studies were slaughtered for the determination of carcass characteristics (carcass and organ weights, gut organ digesta pH and gastro-intestinal length measurements). Dietary threonine levels used in this experiment affected ($P<0.05$) feed intake, growth rate, live weight, metabolisable energy (ME) intake, nitrogen retention, feed conversion ratio and gut organ weights and lengths of unsexed Ross 308 broiler chickens aged 21 days. Dietary threonine level did not affect ($P>0.05$) diet digestibility. Feed conversion ratio, pH of the proventriculus digesta, gut intestine length and caecum length of unsexed broiler chickens were optimized at different dietary threonine levels of 9.6, 8.5, 6.6 and 8.4 g/kg DM, respectively. Dietary threonine levels had an effect ($P<0.05$) on feed intake, diet digestibility, metabolizable

energy, live weight, proventriculus pH values, GIT length, gut organ and carcass organ weights of male Ross 308 broiler chickens between 22 to 42 days of age. Proventriculus and large intestine weights were optimized at different dietary threonine levels of 7.5 and 9.1 g/kg DM feed, respectively. Dietary threonine level did not affect (P>0.05) growth rate, feed conversion ratio of male Ross 308 broiler chickens between 22 to 42 days of age.

It is concluded that dietary threonine levels used in this study affected production performance of younger broilers (Day 1-21) more than that of older birds (Day 22-42). However, production variables were optimized at different dietary threonine levels. This has implication on diet formulation for the chickens and no linear response could be established.

Keywords: Threonine, Broiler chickens, Feed intake, Growth rate, Feed conversion ratio, Live weight, Carcass characteristics.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre.
AOAC	Association of Analytical Chemists.
Ca	Calcium.
^{o}C	Degree centigrade.
Cm	Centimetre.
CP	Crude protein.
DM	Dry matter.
DMI	Dry matter intake.
DTI	Department of Trade and Industry.
d	Day.
FCR	Feed conversion ratio.
Fe	Iron.
G	Gram.
GIT	Gastrointestinal tract.
GLM	General Linear Model.
H	Hour.
N	Nitrogen.
Na	Sodium.
m^2	Meter squared.
Min	Minute.
P	Phosphorus.
%	Percent.
r^2	Coefficient of determination.
RTNTN	Retention.
SAPA	South African Poultry Association.
SAS	Statistical Analysis System.
USA	United States of America.
USDA	United States Department of Agriculture.
WBSF	Warmer Bratzler Shear Force.
Wk	Week.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

There is a global demand for livestock commodities and in particular poultry meat (FAO, 2004; Steinfeld *et al.,* 2006). Feed is the backbone of profitable broiler production and, on an average, it accounts for about 80-90% of the total cost of production (NAFIS 2017). Poultry meat is an important source of protein in the world (Boer *et al.*, 2001). Adequately balanced nutrition is vital to the health, fertility and optimal performance of chickens. Higher growth rates in chickens require diets high in digestible energy and protein, which makes broiler feed very expensive. To minimize costs and increase feed efficiency, modern broiler diets are formulated on the basis of digestible amino acids. The provision of quality protein devoid of any essential amino acids deficiency is particularly critical in the early nutrition of young chicks (Dibner, 2003). In order to maximize performance, poultry diets must contain the correct balance of the essential nutrients required to meet the nutritional demand of the chicken. Threonine requirement levels for optimal productivity of Ross 308 broiler chickens are changing due to breed improvements (Kidd *et al*., 2004). Thus, there has been a lot of improvement in chicken productivity through efficient breeding but relatively less on nutrition (Tallentire *et al.,* 2016). Threonine is a major component of body protein and plays an important role as a precursor of lysine and serine (NRC, 1994). These amino acids are essential for growth and meat tenderness (Ojano-Diranin and Waldroup, 2002).

1.2 PROBLEM STATEMENT

Genetic selections of broiler chickens emphasize improvements in growth (Wang *et al*., 2012). However, such improvements have resulted in alterations at the tissue level in terms of leaner or fatter carcasses, leading to different nutrient requirements and relationships (NRC, 1994). This requires that dietary nutrients match broiler chicken requirements for maintenance and tissue accretion in order to obtain optimal productivity. Thus, threonine deficiency leads to poor growth and meat tenderness in chickens (NRC, 1994). Factors such as dietary crude protein, breed, sex and age of the chicken can affect threonine requirements. Results of the effect of dietary threonine level on productivity of broiler chickens have been variable and not conclusive, varying from 5.8 to 7.9 g/kg DM (NRC, 1994; Wang *et al*., 2012).

1.3 RATIONALE

Carcass weight and composition of chickens are receiving considerable attention. There is emphasis on increasing meat yield, especially breast meat (Wang *et al*., 2012). Poultry are not capable of synthesizing threonine *de novo* which makes it a nutritionally essential amino acid (NRC, 1994). According to Zaghari *et al*. (2011), threonine highly affects the development and adequate functioning of broiler chicken intestines since it is the main amino-acid in mucins. There is evidence that threonine deficiencies lead to poor productivity and increased susceptibility to infection (Richard, 2005). However, results of the effect of dietary threonine level on productivity of chickens have been variable and inconclusive, requirements range from 5.8 to 7.9g/kg DM (Kidd and Kerr, 1996).

Chickens that are fed on a diet with less than the required threonine level have poor growth rate, poor feed conversion ratio (FCR) and lower carcass yield (NRC, 1994; Kidd *et al*., 1999; Kidd *et al*., 2004). It is, therefore, important to provide sufficient amounts of threonine for improved Ross 308 chicken breeds to support the needs of tissue maintenance and accretion while minimising any excesses (Rehman *et* al., 2012).

1.4 Aim

The aim of this study was to determine dietary threonine levels for optimal productivity and carcass characteristics of Ross 308 broiler chickens.

1.5 Objectives

The objectives of this study were to determine:

i. the effect of dietary threonine level on feed intake, digestibility, growth rate and carcass characteristics of Ross 308 broiler chickens aged one to 42 days
ii. dietary threonine levels for optimal responses in feed intake, growth rate and carcass characteristics of Ross 308 broiler chickens aged one to 42 days to dietary threonine level.

1.6 Hypotheses

i. Dietary threonine level had effect on feed intake, growth rate, digestibility and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.

ii. There are no optimal responses in feed intake, digestibility and carcass characteristics of Ross 308 broiler chickens aged one to 42 days to dietary threonine level.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Genetic selection of broiler chickens emphasizes improvements in growth (Wang *et al*., 2012). However, such improvements have resulted in alterations at the tissue level in terms of leaner or fatter carcasses, leading to different nutrient requirements (NRC, 1994). This requires that dietary nutrients match broiler chicken requirements for maintenance and tissue accretion in order to obtain optimal productivity. Threonine is a major component of body protein and plays an important role as a precursor of lysine and serine (NRC, 1994). These amino acids are essential for growth and meat tenderness (Ojano-Diranin and Waldroup, 2002). Factors such as dietary crude protein, breed, sex and age of the chicken can affect the threonine requirements. Results of the effect of dietary threonine level on productivity of broiler chickens have been variable and not conclusive, varying from 5.8 to 7.9 g/kg DM (NRC, 1994; Wang *et al*., 2012).

2.2. Broiler chicken production

There is a global demand for livestock commodities and poultry meat (FAO, 2004; Steinfeld *et al.,* 2006). Chickens are the most abundant and widely kept livestock species in the world (Moreki *et al*., 2010). Chickens have the advantages of having quick returns on investment and relatively simple management practices with numerous market outlets for their products. Ndirangu *et al*. (2010), indicated that poultry contributes to farmers' income. In low-income developing countries, such as South Asia and sub-Saharan Africa, meat is less frequently consumed and less often available as a protein source in diets (FAO, 2012). A survey showed that 34% of the population in South Asia and 59% of the population in sub-Saharan Africa obtained their energy from staple foods such as cereals, grain legumes and starchy roots (Smith and Wiseman, 2007). Some of these staple foods are deficient in the essential amino acids that are required by human beings, for example lysine, threonine, sulphur-bearing amino acids (methionine and cysteine), and occasionally tryptophan, which may lead to protein deficiency (Juliano, 1993).

Poultry meat, is currently one of the best sources of animal protein for low-income populations because it is an affordable and accessible source of protein with low fat content and limited religious restrictions (Smith and Wiesman, 2007; Association of Poultry Processors and Poultry Trade in the EU (AVEC), 2016). As a result, the global

consumption of poultry meat is greater than that of other animal species (Belova *et al*., 2012; Pandurevic *et al*., 2014; Rural Industries Research and Development Corporation (RIRDC), 2014). In addition, growth of the poultry industry in developing countries provides opportunities for employment and improves the economy (Smith and Wiseman, 2007).

In sub-Saharan Africa, the largest producer of chicken meat is South Africa with a consumption of 37.47 kg per capita per year in 2014 (South African Poultry Association (SAPA), 2016). The dominance of chicken meat in the South African meat market is due to similar factors to those driving chicken demand globally and the price of chicken meat is generally lower than that of other animal species (Department of Agriculture, Forestry and Fisheries (DAFF), 2013).

2.3 Nutritional requirements of broiler chickens

Modern broilers can potentially attain 2 kg of body weight by consuming 3 kg of feed within 5 weeks (Choct, 2009). Genetic selection and a nutritionally balanced diet are the main drivers of the faster growth of broilers (Havenstein *et al*., 2003). Nutrient recommendations for broiler feeds are usually appropriate to maximize growth. However, optimum dietary amino acid levels change with the production goal, such as the optimization of growth, breast meat yield, or feed conversion. For instance, optimum amino acid levels for breast meat production have proved to be higher compared with those for the whole carcasses or weight gain and seem to also be dependent on broiler chicken genetics (Schutte and Pack, 1995). According to McDonald *et al*. (2011), nutrition plays a vital role in influencing growth in livestock. Carbohydrates, fats and proteins that the chicken utilizes as sources of energy are essential requirements for growth. Feed formulation based on digestible amino acids has been shown to increase weight gain and feed intake and improve body composition in broiler chickens (Rostagno *et al*., 1995). Reduction in dietary protein levels is known to reduce chicken's performance, meat yields (Temim *et al*., 1999) and immune responses (Rama Rao *et al*., 1999) in chickens. Excess or imbalanced protein is reported to increase the dietary requirement of threonine, which helps in uric acid synthesis as a precursor for glycine (Baker 1985).

Kidd and Kerr (1996) and Hussein *et al*. (2001) reported that threonine is the most limiting amino acid in low crude protein broiler chicken diets since threonine

requirement depends on crude protein content of the diet (Ciftci and Ceylan, 2004). The beneficial effects of threonine supplementation to low protein diets may vary compared to the optimal protein diets. Information on the effects of threonine supplementation to low crude protein diets on performance, carcass yields (Mack *et al*., 1999) and immunity (Maroufyan *et al*., 2010) is very limited. Establishing the optimum requirement of threonine in broiler chicken diets will help poultry nutritionists to formulate diets that meet the nutrient requirements of these birds.

There are about 500 types of essential molecules known as amino acids in all living organism and 20 of them are genetically encoded (Walsh *et al*., 2013). Out of these 20 amino acids, 10 are classified as essential, meaning that they cannot be synthesized at all or rapidly enough to meet metabolic requirement, and must be supplied in the diets for a maximum growth performance. Out of these 10 essential amino acids, methionine and lysine are considered as the first two limiting amino acids for broilers (Corzo *et al*., 2007), whereas threonine is ranked the third limiting amino acid (Kidd and Kerr, 1996). Threonine is very important in the synthesis and maintenance of animal body proteins and contains 11.7% nitrogen (Kidd and Kerr, 1996). Threonine requirement of broilers depends on various factors such as age of the birds, dietary crude protein level and core ingredients in the diet (Barkley and Wallis, 2001). National Research Council (1994) recommended values for total dietary threonine were 0.80% for starter (0 to 21 days), 0.74% for grower (22 to 42 days), and 0.68% for finisher (43 to 56 days) periods. However, all the dietary essential amino acids need to be expressed as the percentages of the lysine in the diets (NRC, 1994).

Strakova *et al*. (2003) noted that the most important components of poultry meat are mainly proteins with a high content of essential amino acids, especially arginine, leucine, isoleucine, methionine and valine in comparison to pork and beef meat. Methionine, cysteine and threonine are essential for body maintenance for chickens aged 6 to 8 weeks and the recommended value is 0.68% for metabolic processes, cell synthesis and renewal (Kidd *et al*., 2004). Threonine is important not only for protein deposition, but also for mucin production and digestive processes (Ball *et al*., 1999). Mack *et al*. (1999) and Kidd (2000) reported that threonine requirement for feed conversion is higher than that for weight gain in chickens.

Table 2.01 Dietary nutrient levels for broiler chickens

	Broiler Starter	Broiler grower	Broiler finisher
ME (MJ/kg DM)	12.7	13.2	13.4
Crude protein (g/kg)	220-250	210-230	190-230
Digestible amino acids (g/kg) [b]			
Arginine	13.1	11.4	10.2
Isoleucine	8.5	7.5	6.7
Lysine	12.7	11	9.7
Methionine	4.7	4.2	3.8
Methionine+Cystine	9.4	8.4	7.6
Threonine	8.3	7.3	6.5
Tryptophan	2	1.8	1.6
Valine	9.5	8.4	7.5
Major minerals (g/kg)			
Calcium	10.5	9	8.5
Phosphorus (average)	5	4.5	4.2
magnesium	3	2.5	2.5
sodium	2	2	1.8
potassium	7	6.5	6.5
Trace minerals (mg/kg; supplementary levels)			
Copper	16	16	16
Iodine	1.25	1.25	1.25
Iron	40	40	40
Manganese	120	120	120
Zinc	100	100	100
Selenium	0.3	0.3	0.3
Vitamins (iu/kg; supplementary levels) [a]			
A	12 000	10 000	10 000
D_3	5 000	5 000	4 000
E	75	50	50
Vitamins (mg/kg; supplementary levels) [a]			
K	3	3	2
Thiamin	3	2	2
Riboflavin	8	6	5
Choline	1 600	1 500	1 400
B_{12}	0.016	0.016	0.01

[a]Supplementary trace minerals and vitamin levels required differ according to whether the basal diet is wheat or maize.

[b]For broilers = True whole tract digestibility and for laying hens = Standardised ileal digestibility.

Source: McDonald *et al*. (2011)

2.4 Biochemical functions of threonine

Besides its utilization for protein synthesis, threonine is involved in other biological functions such as the maintenance of gut integrity and immunity. Thus, a deficiency in threonine leads also to disorders in the digestive physiology which can increase the frequency of digestive problems. As a consequence, threonine requirement varies depending on the relative importance of these different functions. It is, therefore, important to determine the threonine requirement which corresponds to each physiological stage to improve the balance and efficiency of feeds. Threonine participates in protein synthesis, and its catabolism generates many products important in metabolism (Lemme, 2003). Threonine is an alpha amino acid with the chemical formula $HO_2CCH(NH_2)CH(OH)CH_3$ (Figure 2.1). This essential amino acid is classified as polar. Threonine can reside both within the interior of a protein, or on the protein surface. Together with serine, threonine is one of the two proteinogenic amino acids bearing an alcohol group (Lehninger *et al*., 2000). Threonine is quite common in protein functional centres. The hydroxyl group is fairly reactive, being able to form hydrogen bonds with a variety of polar substrates. A common role for threonine within intracellular proteins is phosphorylation (Betts and Russell, 2003). Protein kinases frequently attach phosphates to threonine in order to facilitate the signal transduction processes.

Threonine can be converted to pyruvate or to alpha-ketobutyrate and eventually to succinyl-CoA, suggesting an association with the citric acid cycle (Balch, 2000). It is one of the amino acids that can be phosphorylated, which is a major mechanism by which cells control various signalling pathways. In addition, it is required for the body to synthesize two non-essential amino acids, glycine and serine, both of which play important roles in various physiological functions (Lehninger *et al*., 2000).

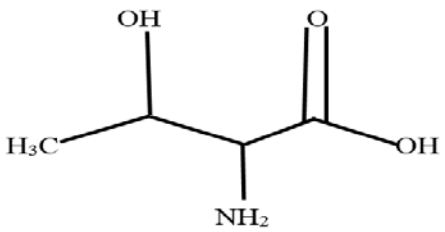

Figure 2.1: The structure of threonine (Lehninger *et al*., 2000)

2.5 The effect of threonine on chicken productivity

Threonine is needed for optimal immune response and gastrointestinal mucine production (Kidd, 2000). It is reported to improve the live weight gain of heat-stressed broiler chickens (Kidd, 2000). Adequate digestible threonine levels are required to support optimum growth since it serves an important role as a precursor of lysine and serine which are important components of meat (Ojano and Waldroup, 2002). Kidd *et al*. (2004), recommended a dietary digestible threonine requirement of 0.65% to achieve optimum weight gain and breast meat weight in boiler chickens aged 3 to 6 weeks. According to Kidd *et al*. (2000), dietary threonine requirement for optimum live weight and breast meat yield is 0.67% of the diet for male broiler chickens aged 6 to 8 weeks in a thermos-neutral environment (25 to 32 ℃). Webel *et al*. (1996), estimated the dietary threonine requirement of male broiler chickens aged 6 to 8 weeks to be 0.60% for optimum live weight gain. Increased dietary threonine level improved nitrogen retention in broiler chicks (Dozier, 2001). However, Ojano-Diranin and Waldroup (2002) suggested that the modern rapidly growing broiler chicken may have threonine requirement greater than the 0.74% that is generally recommended by NRC (1994). It can be concluded that threonine requirement values for broiler chickens vary greatly.

Mack *et al*. (1999) and Kidd (2000) reported that threonine requirement for feed conversion is higher than that for weight gain in chickens. For instance, optimum amino acid levels for breast meat production have proved to be higher compared with those for the whole carcass or weight gain and seem to also be dependent on broiler chicken genetics (Schutte and Pack, 1995). There is evidence for broiler chickens that adequate threonine levels are needed to support optimum growth rates and meat tenderness, and reduce mortality rates (Kidd *et al*., 1999). Feed formulation based on digestible amino acids has been shown to increase weight gain and feed intake and improve body composition in broiler chickens (Rostagno *et al*., 1995).

Kidd and Kerr (1996) and Hussein *et al*. (2001) reported that threonine is the most limiting amino acid in low crude protein broiler chicken diets. Threonine requirement depends on crude protein content in the diet (Ciftci and Ceylan, 2004). The beneficial effects of threonine supplementation to low protein diets may vary compared to the optimal protein diets. Information on the effects of threonine supplementation to low crude protein diets on performance, carcass yields (Mack *et al*., 1999) and immunity (Maroufyan *et al*., 2010) is very limited. Results of the effect of dietary threonine level

on productivity of broiler chickens have been variable and not conclusive; varying from 5.8 to 7.9 g/kg DM (NRC, 1994; Wang *et al*., 2012). Establishing the optimum requirement of threonine in Ross 308 broiler chicken diets will also help poultry nutritionists to formulate diets with optimum economic and ecological benefits.

Table 2.02 Dietary threonine requirements (% of diet) for optimum feed intake, weight gain, feed conversion ratio and breast muscle weight of broiler chickens at different ages

Age	Intake	Weight gain	FCR	Breast muscle	Authors
1-3 Weeks	-	-	0.87	-	NRC(1994)
1-3 Weeks	-	0.73	0.77	-	Thomas(1986)
2-4 Weeks	0.69	-	-	-	Thomas(1986)
2-4 Weeks	-	-	0.76	-	Kid *et al.* (2004)
2-6 Weeks	-	-	-	0.79	Corza *et al.* (2007)
3-6 Weeks	-	0.65	-	0.65	Kidd *et al.* (2004)
4-5 Weeks	0.74	0.74	0.74	-	NRC(1994)
4-6 Weeks	-	0.75	0.75	0.75	Kidd and Kerr(1997)
6-7 Weeks	-	0.60(Males)	-	-	Webel *et al.* (1996)
6-7 Weeks	-	0.67(Males)	-	0.67	Kidd *et al.* (2004)
6-8 Weeks	-	0.68	0.68	0.68	NRC(1994)

Growth performance is an essential parameter to evaluate the effectiveness of feed offered to broiler chickens. Different environmental conditions, also, influence growth performance of broilers, depending on the dietary threonine level (Kidd *et al*., 2003). Threonine is an essential amino acid that promotes normal growth by helping to maintain the proper protein balance in the body. It also supports cardiovascular, liver, central nervous and immune systems. Threonine helps keep connective tissues and muscles, throughout the body, strong and elastic, including the heart (Balch, 2000). Threonine, also, has effect on immunity as it plays a major role in achieving maximum growth performance of broilers. The immune status of the broilers improves, with an improvement in the function of immune organs (Corzo *et al*., 2007; Zhang *et al*., 2016). Under unhygienic environmental conditions, dietary threonine requirements are

increased to sustain the maintenance necessities in the gut mucosa (Corzo *et al*., 2007) and to enhance immunity (Bhargava *et al*., 1971).

Chee *et al*. (2010) reported that an excess intake of threonine can increase the numbers of ileal lactobacilli and lactic acid bacteria in broilers. Additionally, it has been reported that dietary threonine can reequilibrate the gut microbiota in the animals under stress (Faure *et al*., 2006; Wils-Plotz et al., 2013; Trevisi *et al*., 2015). A higher level of dietary threonine supplementation can reduce ileal *Eimeria maxima* counts in young broilers challenged with *Eimeria maxima* (Wils-Plotz *et al*., 2013).

2.6 Tenderness, flavourness and juiciness of chicken meat

Palatability can be defined by three characteristics. These are tenderness, juiciness and flavour or odour. In most countries, consumers prefer poultry meat tenderness (Warris, 2000). Juiciness depends on the amount of water retained in a cooked meat product. Juiciness increases flavour, helps soften meat making it easier to chew, and stimulates saliva production in the mouth. Water retention and lipid content determine juiciness. Marbling fat around edges helps hold in water. Water losses are from evaporation and drip losses. Meat aging can increase water retention and therefore increases juiciness.

Tenderness has been linked to several factors, such as the animal's age, sex or the muscle location. One important way to tenderize meat is by aging. Carcasses are aged by holding them at refrigeration temperatures for extended periods of time after slaughter and initial chilling. These perceptions rely on smell and on the sensations of salty, sweet, sour and bitter. Meat flavour is affected by type of species, diet, cooking method and method of preservation (Zhang *et al*., 2005).

Taste, texture, juiceness, appearance and adour are five main characteristics that contribute to the overall eating quality of meat. Among these characteristics texture is probably considered to be the most important attribute by the average consumer (Dransfield, 1994; Chrystall, 1994). For the consumer, from a sensory point of view, the most important thing is tenderness of the meat. It increases the enjoyment while eating (Chrystall, 1994).

2.6.1 Tenderness

Meat tenderness and juiciness are positively correlated with the proportion of fat in the carcass (Wood, 1990; Bruns *et al*., 2004). Marbling fat has no direct effect on meat tenderness (Renand *et al*., 2003; Thompson, 2004). However, it plays an important role in meat juiciness and overall eating satisfaction. In fact, marbling leads to greater palatability in panel scores (McPeake, 2001) and lower shear force values (Dolezal *et al*., 1982). Carcasses with higher marbling content also have a higher subcutaneous and intermuscular fat content, thus insulating the muscles during chilling and preventing the phenomenon of cold shortening. Fatter carcasses undergo a faster drop in pH, which is associated with more tender meat; and slower cooling of fatter carcasses contributes to an increase in the activity of ageing enzymes, leading to greater tenderness (Wood, 1997).

Consumer acceptance of chicken meat depends on its eating quality, which is influenced by a number of factors ranging from the physical and chemical to the histological properties and processing and handling of meat (Alvarado and Sams, 2004). One of the textural properties, tenderness has been noted as the most important factor determining quality of meat products (Savell *et al*., 1989). Hoffmann (1995) defined meat quality as the sum of all sensory, nutritionally, hygienic-toxicological and processing technological properties of the meat. Thus, the physical criteria after slaughter are of importance (Ristic *et al*., 2012). Tenderness of meat is a physiological property, which can be influenced by various factors like breeding, husbandry, feeding, fattening age, slaughter technology, cooling, storing and not least by the thermic treatment (Ristic *et al*., 2012). It comprises different material properties, like bite characteristics, succulence and toughness.

The toughness can be examined in different ways: biochemical, physical, mechanical and sensory (Sikes *et al*., 2010). Meat tenderness originates in structural and biochemical properties of skeletal muscle fibres, especially myofibrils and intermediate filaments, the endomysium and perimysium, which are composed of collagen fibrils and fibres. Meat tenderness is a dimensional attribute and is described in several stages; partial compression, first bite, chew down and residual (Meilgaard *et al*., 2016).

Tenderness also decreases as animals mature because of the cross-linking of collagen (Fletcher, 2002). The tenderness of meat is the sum of mechanical strength of skeletal muscle tissue and its weakening during the post-mortem aging of meat

(Takahashi, 1996). Tenderness tends to be influenced by contraction state of myofibrillar proteins and maturity of connective tissues (Fletcher, 2002). Myofibrillar contribution to the meat tenderness depends on the extent of shortening during rigour mortis development and proteolysis during conditioning (Warris, 2000). Connective tissues toughness is often referred to as background toughness because the tissues hardly change during the standard length of meat storage post-mortem (McCormick, 1994).

The degree to which meat is cooked is very important in determination of meat tenderness (Warris, 2000). Meat toughness is caused by excessive contraction during rigour mortis development after aging period, supposedly by proteolysis, non-enzymatic degradation of the cytoskeleton and the weakening of actin and or myosin interactions (Takahashi, 1996). This is determined by the pre- and post-slaughter effects on the carcasses.

2.6.2 Flavourness

Flavour and odour are closely related. Flavour is generally linked to water-soluble materials, and odour is related to fat-soluble volatile elements. If the meat smells unpleasant, it is mostly related to the quality of the meat. Meat flavour is another quality attribute that consumers use to determine the acceptability of chicken meat. Taste and odour contribute to the flavour of poultry meat, and it is difficult to differentiate between the two during consumption. Few factors affect poultry meat flavour during production and processing. Apart from juiciness, flavour and colour are the main eating quality characteristics that influence the consumers overall judgment of quality (Wood *et al*., 1995).

Thus, it is not only difficult to produce a flavour defect, but it is difficult to enhance flavour during production and processing (Fletcher, 1999). Minor effects in meat flavour are related to bird strain, environmental conditions, diet, chilling, scalding temperatures, product packaging and storage; however, these effects are too minimal for consumers to notice them (Fletcher, 2002). Age of the bird at slaughter affects the flavour of the meat. A very large number of compounds have been identified as potential contributors to meat flavour. However, it is probable that only a relative few of these effects are important. A compound’s contribution to flavour depends on two things: first, how much is produced and second, the odour threshold (Lawrie, 1998).

Consumers generally decide to purchase meat based on its appearance. The colour of the meat greatly affects its saleability. Meat water-holding capacity is also important to the consumer. It can be said that appearance and technological characteristics are connected. The importance of water-holding capacity can be explained in three ways: firstly, poor water holding-capacity can be connected to the appearance of the meat. Water holding-capacity is obvious to the consumer when examining the packaging in the retail stores. Poor water holding-capacity results in the drip remaining in the package, which also results in a negative appearance of the meat. Secondly, the drip loss is connected to the weight of the meat. In processed meat, poor water holding-capacity may reduce water retention and, therefore, yield of product is reduced. Finally, the juiciness of the meat after cooking is also affected by the water holding-capacity. Poor water holding-capacity meat may be dry, or taste may be negatively affected. Besides colour and water holding-capacity there is also a relationship between appearance and marbling fat. This is also an important factor for determining appearance of the meat (Warris, 2000).

2.6.3 Juiciness

Juiciness of the meat is mainly related to the water-holding capacity of the meat or low marbling fat level. Consumers consider tenderness and juiciness to be the most important quality attributes of fresh meat and meat products (Xiong, 2005). Increased water and fat content at the time of consumption are generally associated with increased juiciness. Decreased water binding capacity and loss of moisture or fat through drip or cooking would decrease juiciness. Good-quality meat is juicier than that of poor quality, the difference being at least partly attributable to the higher content of intramuscular fat in the former (Gaddis *et al*., 1950).

There is some suggestion that juiciness reaches a minimum when the pH level of the meat is about 6 (Howard and Lawrie, 1956). This possibly reflects the greater ability of the muscle proteins to bind water in this pH region; but, if this were the entire explanations, juiciness would be expected to decrease still further with even higher pH levels. The process of freezing does not itself affect juiciness (Gaddis *et al*., 1950), there being no difference in this respect between meat which has been chilled or frozen and held for the same length of time.

Juiciness in the cooked meat has two organoleptic components, first is the impression of wetness during the initial chewing, which is due to the rapid release of meat fluids. Second is the sustained juiciness resulting from the stimulatory effect of fat on the salivary glands. In conjunction with tenderness, juiciness accounts for the overall eating quality and consumers may confuse the two factors when making comparisons (Varnam and Sutherland, 1995). Savell and Cross (1988) stated that 'fat may affect juiciness by enhancing the water holding capacity of meat, by lubricating the muscle fibres during cooking, by increasing the tenderness of meat and thus the apparent sensation of juiciness, or by stimulating salivary flow during mastication'.

2.7 Effects of threonine on carcass characteristics of broiler chickens

In broiler chickens, threonine requirement for carcass yield is variable, depending upon age, strain, sex of broiler chickens, crude protein content of feed and proportion of dietary ingredients used (Barkley and Wallis, 2001). The improved carcass characteristics may be due to increased amount of essential amino acids in the diet (Estalkhzir *et al*., 2013). Al-Hayani (2017) reported that broilers fed a diet containing 0.90% of threonine resulted in an enhanced carcass weight by 3.7%, breast weight by 2.3% whereas, thigh weight was reduced by 1.1%. Similarly, El-Faham *et al*. (2017) executed a trial by using two threonine levels of 0.77 and 0.87% in broiler diets. The results revealed that the broiler chickens fed diets containing 0.87% of threonine showed 28% higher breast weight, 1.6% increased drum stick weight and 9.6% reduced thigh weight compared with those fed diet containing 0.77% of threonine. Rezaeipour and Gazani (2014) compared the effect of 0.74 and 0.77% total dietary threonine on carcass characteristics in broiler chickens. It was observed that broiler chickens fed 0.77% total dietary threonine showed increased breast meat by 0.4%.

2.8 Conclusion

Broiler chickens play an important role in the rural households as a source of income and nutrition. The information on the effect of dietary threonine supplementation on feed intake, digestibility, growth rate, mortality and carcass characteristics of broiler chickens is extensive but not conclusive. It is, therefore, important to determine the effect of dietary threonine level on productivity and carcass characteristics of broiler chickens.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was conducted at the University of Limpopo Livestock Unit (latitude 27.55°S and 24.77°E), Limpopo Province, South Africa. The ambient temperatures around the study area range between 20 and 36°C during summer and between -5 and 28°C during winter (Shiringani, 2007).

3.2 Preparation of the poultry house

The poultry house was cleaned properly with water and a disinfectant (Vet GL 20, NTK Company, Polokwane). The house was left for a week after cleaning to break the life cycle of infectious microorganisms. After proper drying, the poultry house was divided into 15 floor pens of $2m^2$ per pen. Fresh saw dust was spread in each pen to a thickness of 7cm. The heating system was provided using 250 watt-infrared lights.

3.3 Acquisition of materials and chickens

Day-old Ross 308 broiler chicks were acquired from Lufafa hatchery, Tzaneen, South Africa. The required ingredients to formulate starter and grower diets were acquired from Voorslagvoere Milling Company in Mokopane, South Africa. House hold disinfectants, medicines, 250 watts infrared lights, feeders and drinkers were acquired from NTK, Polokwane, South Africa.

3.4 Experimental designs, treatments and procedures

The first part of the study was to determine the effect of dietary threonine level on feed intake, growth rate, mortality and carcass characteristics of Ross 308 broiler chickens aged between 1 and 21 days. A total of 150 unsexed day-old chicks were used in a complete randomized design having 5 treatments (6.4, 7.5, 8, 8.5 and 9g of threonine/kg DM feed), replicated three times and having ten chickens per replicate (Table 3.01). Light was provided 24 hours daily while feed and water were provided *ad libitum* throughout the study. The experimental diets were isocaloric and isonitrogenous, but with different threonine levels. All the diets contained 21% crude protein as recommended by NRC (1994). The experimental chicks were fed starter diets formulated from the feed ingredients as indicated in Table 3.02.

Table 3.01 Dietary treatments for Experiment 1

Diet code	Diet description
$UT_{6.4}$	Unsexed Ross 308 broiler chickens on a 21% CP diet having 6.4g of threonine/kg DM

$UT_{7.5}$	Unsexed Ross 308 broiler chickens on a 21% CP diet having 7.5g of threonine/kg DM
$UT_{8.0}$	Unsexed Ross 308 broiler chickens on a 21% CP diet having 8.0g of threonine/kg DM.
$UT_{8.5}$	Unsexed Ross 308 broiler chickens on a 21% CP diet having 8.5g of threonine/kg DM.
$UT_{9.0}$	Unsexed Ross 308 broiler chickens on a 21% CP diet having 9.0g of threonine/kg DM.

Table 3.02. Ingredients and nutrient composition of starter diets for Experiment 1

	Treatment*

Feed ingredient	$UT_{6.4}$	$UT_{7.5}$	$UT_{8.0}$	$UT_{8.5}$	$UT_{9.0}$
Yellow maize (%)	60.00	59.02	59.00	58.98	58.96
Maize gluten 60 (%)	1.1	5.0	5.00	5.00	5.00
Wheat bran (%)	3.2	2.50	2.57	2.65	2.71
Soybean 46 (%)	24.00	24.30	24.20	24.09	24.01
L- lysine Hcl (%)	0.10	0.10	0.10	0.10	0.10
D-L methionine (%)	0.10	0.30	0.30	0.30	0.30
L- Threonine (%)	0.00	0.01	0.06	0.11	0.16
Vitamins + minerals Premix (%)	0.10	0.10	0.10	0.10	0.10
Limestone (%)	1.80	1.80	1.80	1.80	1.80
Salt (%)	0.50	0.50	0.50	0.50	0.50
Monocalcium P (%)	4.35	1.62	1.62	1.62	1.62
Sodium bicarbonate %	0.30	0.30	0.30	0.30	0.30
Oil sunflower (%)	4.45	4.45	4.45	4.45	4.45
Total	100	100	100	100	100
Analysed nutrient composition					
Crude Protein (%)	21	21	21	21	21
Energy (MJ/kg DM)	14	14	14	14	14
Threonine (g/kg DM)	6.4	7.5	8.0	8.5	9.0
ADF (%)	5.71	5.71	4.92	4.43	4.72
NDF (%)	19.07	16.16	13.89	25.86	25.48
Fat (%)	6.89	6.01	6.41	4.91	3.43
Ash (%)	7.73	8.27	8.42	8.22	6.87
Ca (%)	1.02	1.02	1.01	1.01	1.01

*: Dietary treatments contained 6.4, 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM feed.

The second part of the study determined the effect of dietary threonine level on feed intake, digestibility, growth rate, mortality and carcass characteristics of male Ross 308 broiler chickens aged between 21 and 42 days. Seventy-five male chickens were used in a complete randomized design having 5 treatments (6.4, 7.5, 8, 8.5

and 9g of threonine/kg DM feed), replicated three times and having five chickens per replicate (Table 3.03). Light was provided 24 hours daily while feed and water were provided *ad libitum* throughout the experimental period. The experimental diets were isocaloric and isonitrogenous, but with different threonine levels. All the diets contained 20% crude protein as recommended by NRC (1994). The experimental chicks were fed grower diets formulated from the feed ingredients as indicated in Table 3.04.

Table 3.03 Dietary treatments for Experiment 2

Diet code	Diet description
$MT_{6.4}$	Male Ross 308 broiler chickens on a 20% CP diet having 6.4g of threonine/kg DM
$MT_{7.5}$	Male Ross 308 broiler chickens on a 20% CP diet having 7.5g of threonine/kg DM
$MT_{8.0}$	Male Ross 308 broiler chickens on a 20% CP diet having 8.0g of threonine/kg DM.
$MT_{8.5}$	Male Ross 308 broiler chickens on a 20% CP diet having 8.5g of threonine/kg DM.
$MT_{9.0}$	Male Ross 308 broiler chickens on a 20% CP diet having 9.0g of threonine/kg DM.

Table 3.04. Ingredients and nutrient composition of grower diets for Experiment 2

	Treatment*

Feed ingredient	$UT_{6.4}$	$UT_{7.5}$	$UT_{8.0}$	$UT_{8.5}$	$UT_{9.0}$
Yellow maize (%)	60.54	54.00	54.00	54.00	54.00
Maize gluten 60 (%)	1.1	0.37	2.51	2.35	2.18
Wheat bran (%)	3.00	3.24	3.00	3.00	3.00
Soybean 46 (%)	27.26	30.14	29.02	29.11	29.21
L- lysine Hcl (%)	0.10	0.23	0.10	0.10	0.10
D-L methionine (%)	0.10	0.64	0.50	0.50	0.50
L- Threonine (%)	0.00	0.01	0.04	0.09	0.14
Vitamins + minerals	0.10	0.15	0.15	0.15	0.15
Premix (%)					
Limestone (%)	1.76	1.76	1.77	1.77	1.77
Salt (%)	0.30	0.44	0.44	0.44	0.44
Monocalcium P (%)	1.64	1.61	1.61	1.61	1.61
Sodium bicarbonate %	0.10	0.10	0.10	0.10	0.10
Oil sunflower (%)	4.00	7.30	6.75	6.77	6.79
Total	100	100	100	100	100
Analysed nutrient composition					
Crude Protein (%)	20	20	20	20	20
Energy (MJ/kg DM)	16	16	16	16	16
Threonine (g/kg DM)	6.4	7.5	8.0	8.5	9.0
ADF (%)	5.00	5.00	4.63	5.38	5.81
NDF (%)	17.04	17.40	17.00	17.30	17.10
Fat (%)	4.51	4.95	5.37	5.46	5.23
Ash (%)	6.45	6.77	6.66	6.62	6.32
Ca (%)	1.01	1.02	1.01	1.00	1.01

*: Dietary treatments contained 6.4, 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM feed.

3.5 Data collection

The initial live weights of the chicks were measured at the beginning of the experiment. Average live weight per bird was measured at weekly intervals by weighing the chickens in each pen and dividing the total live weight by the number of birds in the

pen. These live weights were used to calculate growth rate of the chickens. Daily feed intake was measured by calculating the difference between weight of feed offered and weight of feed leftover and the difference was divided by the total number of chickens in the pen. Feed conversion ratio per pen was calculated as total feed consumed divided by the weight gain of the birds in that pen (McDonald *et al.,* 2010).

Apparent digestibility was determined when the chickens were aged 14 to 21 days and 35 to 42 days for Experiments 1 and 2, respectively. Apparent digestibility trials were conducted in specially designed metabolic cages equipped with separate feed and water troughs. One bird was randomly selected from each replicate and transferred to the metabolic cage for the measurement of apparent digestibility. A three-day acclimatization period was allowed prior to a four-day total faecal collection period. Droppings voided by each bird were collected daily at 8.00 hours.

The chickens were slaughtered at the ages of 21 and 42 days for Experiments 1 and 2, respectively, following ethical standards as recommended by the University of Limpopo Animal Research Ethics Committee (AREC/12/2017: PG). Chickens were slaughtered for the determination of carcass characteristics (carcass and organ weights, gut organ digesta pH and gastro-intestinal length measurements). Before the slaughtering, each chicken was weighed using an electronic weighing balance. The dead chickens were then put inside a bucket containing hot water for few seconds and they were then taken out for defeathering by hands. The carcasses were cut open at the abdominal site and the digestive tracts were removed from the abdominal cavities of the chickens. The pH of gizzard, crop, ileum, caecum and large intestine digesta were measured. The digesta pH was measured at each segment using an electonic pH meter prior to the emptying of the digesta for weight measurement.

Two chickens were slaughtered per replicate. Carcass weight of each chicken was measured only at the age of 42 days. Gastrointestinal tract, small intestine, caeca and large intestine lengths were determined using a tape measure. The pH of gut contents (crop, proventriculus, gizzard, ileum, caecum, and colon) were measured using a digital pH meter (Crison, Basic 20 pH meter). Breast, drumstick, thigh, crop, proventriculus, gizzard, small intestine, caeca and large intestine weights were measured using an electronic weighing balance.

3.6 Chemical analysis

Dry matter of the feed, feed refusals and meat samples were determined by drying the sample in the oven for 24 hours at the temperature of 105^0C. Ash content of the feeds, faeces, feed refusals and meat samples were determined by ashing the sample at 600^0C in a muffle furnace overnight. Nitrogen contents of the samples were determined by Kjedahl method (AOAC, 2010). Threonine, fatty acid and other amino acid contents of feeds and meat were determined by iron-exchange chromatography at the University of Limpopo. Gross energy values for feeds and faeces were determined using an adiabatic bomb calorimeter according to the method previously described by Association of Analytical Chemists (AOAC) (2000) at the University of Limpopo Animal Nutrition Laboratory. A full analysis for faeces and feeds (diet composition) was performed at the Pietermartizburg laboratory, Kwa-Zulu Natal, South Africa.

3.7 Sensory evaluation

Meat samples which were previously frozen at -40°C for 4 days were thawed for 7 hours at room temperature prior to cooking. The breast meat was prepared, and the skin was left on the meat samples. The method adopted by Pavelková *et al.* (2013) was used for sensory evaluation of the meat. The following sensory attributes were evaluated by the sensory panel: tenderness, juiciness and flavour of meat samples. The sensory panel consisted of 20 trained panellists. 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. The five-point ranking scale scores used are as indicated in Table 3.05. Nothing was added to the meat samples so as not to affect taste. An oven set at 105ºC was allowed to preheat prior to cooking. The meat samples were put in trays and they were covered with aluminium foil to prevent water loss. Thereafter, the trays with meat were put in an oven for approximately 60 minutes and the meat samples were turned after every 10 minutes. Samples were cut into small 5cm cubic pieces and served immediately after cooking. The individual breast meat was selected for sensory evaluation because of ease of handling.

Table 3.05 Evaluation scores used by the sensory panel

Sensory attribute

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

Source: Pavelková *et al.* (2013)

3.8 Data analysis

Effect of threonine level on feed intake, digestibility, growth rate, mortality rate, feed conversion ratio and carcass characteristics of Ross 308 broiler chickens were analysed using General Linear Model (GLM) procedures for the statistical analysis of variance (SAS, 2008) to detect dietary treatment effects. Where there were significant differences ($P<0.05$), Turkey's honestly significant difference test (HSD) was used for mean separation. The model, $y_{ij} = \mu + T_i + e_{ij}$, was applied in Experiments 1 and 2, where Y_{ij} was the observation on feed intake, feed conversion ratio, growth rate, digestibility, carcass characteristics and mortality due to dietary treatment effects; μ was the overall mean; T_i was the i^{th} effect of dietary threonine level and e_{ij} was the residual effect (error).

The responses in optimal feed intake, feed conversion ratio, growth rate and carcass characteristics and meat tenderness, flavour and taste changes to dietary threonine level were modelled using the following quadratic equation:

$$Y = a + b_1x + b_2 x^2 + e$$

Where Y = optimum feed intake, feed conversion ratio, growth rate, carcass characteristics and mortality; a = intercept; b = coefficients of the quadratic equation; × = dietary threonine level; $-b_1/2b_2$ = x value for optimal response; e = error.

CHAPTER FOUR

RESULTS

4.1 Broiler chickens aged one to 21 days

Results of the nutrient composition of the experimental diets are presented in Table 4.1. The experimental diets were isocaloric and isonitrogenous, but with different threonine levels. All the diets contained 21% crude protein and 16 MJ of energy per kg DM as recommended by NRC (1994) for broiler chickens aged 1 to 21 days.

Table 4.1 Diet composition (% except MJ/kg DM for energy and g/kg DM for threonine) for Experiment 1

	Treatment[#]				
Nutrient	$UT_{6.4}$	$UT_{7.5}$	$UT_{8.0}$	$UT_{8.5}$	$UT_{9.0}$
DM	90	90	90	90	90
CP	21	21	21	21	21
Energy	16	16	16	16	16
ADF	5.71	5.71	4.92	4.43	4.72
NDF	19.07	16.16	13.89	25.86	25.48
Fat	6.89	6.01	6.41	4.91	3.43
Ash	7.73	8.27	8.42	8.22	6.87
Ca	1.02	1.02	1.01	1.01	1.01
Threonine	6.4	7.5	8	8.5	9

: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

Results of the effects of threonine level on feed intake, digestibility, growth rate, feed conversion ratio (FCR), live weight, metabolisable energy intake and nitrogen retention of unsexed Ross 308 broiler chickens aged one to 21 days are presented in Table 4.2. The dietary threonine level affected ($P<0.05$) feed intake of unsexed Ross 308 broiler chickens in Weeks 1, 2 and 3. In Week 1, unsexed Ross 308 broiler chickens on a diet containing 6.4 g of threonine per kg DM had higher ($P<0.05$) feed intakes than those on a diet containing 8.5 g of threonine per kg DM. However, unsexed Ross 308 broiler chickens on diets containing 6.4, 7.5, 8.0 or 9.0 g of threonine per kg DM had similar ($P>0.05$) feed intakes in Week 1. Similarly, unsexed Ross 308 broiler chickens on diets containing 7.5, 8.0 or 9.0 g of threonine per kg DM had the same ($P>0.05$) feed intakes. Unsexed Ross 308 broiler chickens on a diet containing 6.4 g of threonine per kg DM had higher ($P<0.05$) feed intakes than those on diets containing 8.0, 8.5 or 9.0 g of threonine per kg DM during Week 2. Similarly, broiler chickens on a diet containing 9.0 g of threonine per kg DM had higher ($P<0.05$) feed intakes than those on a diet containing 8.5 g of threonine per kg DM. However, broiler chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had the same ($P>0.05$) feed intakes during Week 2. Broiler chickens on diets containing 7.5, 8.0 or 9.0 g of threonine per kg DM had similar ($P>0.05$) feed intakes during Week 2. Similarly, unsexed Ross 308 broiler chickens on diets containing 8.5 or 8.0 g of threonine per kg DM had the same ($P>0.05$) feed intakes in Week 2. Broiler chickens on diets containing 6.4 or 9.0 g of

threonine per kg DM had higher (P<0.05) feed intakes than those on diets containing 7.5, 8.0 or 8.5 g of threonine per kg DM in Week 3. However, broiler chickens on diets containing 6.4 or 9.0 g of threonine per kg DM had similar (P>0.05) feed intakes. Similarly, broiler chickens on diets containing 7.5, 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) feed intakes. Dietary threonine level on unsexed Ross 308 broiler chickens aged 1 to 21 days had no effect (P>0.05) on diet DM, CP, NDF, ADF, fat and ash digestibility values (Table 4.2).

The present study indicates that dietary threonine level had effect (P<0.05) on metabolisable energy (ME) intakes and nitrogen retention values of unsexed Ross 308 broiler chickens aged three weeks (Table 4.2). Broiler chickens on diets containing 8.0 or 9.0 g of threonine per kg DM had higher (P<0.05) metabolisable energy intakes than those on diets having 6.4, 7.5 or 8.5 g of threonine per kg DM. Broiler chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had higher (P<0.05) metabolisable energy intakes than those on a diet having 8.5 g of threonine per kg DM. However, chickens on diets having 6.4 or 7.5 g of threonine per kg DM had similar (P>0.05) metabolisable intakes. Similarly, chickens on diets having 8.0 or 9.0 g of threonine per kg DM had similar (P>0.05) metabolisable energy intakes. Unsexed Ross 308 broiler chickens on diets containing 8.0 or 9.0 g of threonine per kg DM had higher (P<0.05) nitrogen retention values than those on diets having 7.5 or 8.5 g of threonine per kg DM. Broiler chickens on a diet containing 6.4 g of threonine per kg DM had higher (P<0.05) nitrogen retention than those on a diet having 8.5 g of threonine per kg DM. However, chickens on diets having 6.4 or 7.5 g of threonine per kg DM had similar (P>0.05) nitrogen retention values. Chickens on diets having 6.4, 8.0 or 9.0 g of threonine per kg DM had similar (P>0.05) nitrogen retention values. Similarly, chickens on diets having 7.5 or 8.5 g of threonine per kg DM had the same (P>0.05) nitrogen retention values.

Dietary threonine level had no effect (P>0.05) on growth rates of unsexed Ross 308 broiler chickens aged one or 3 weeks (Table 4.2). However, dietary threonine level affected (P<0.05) growth rates of unsexed Ross 308 broiler chickens aged two weeks. Chickens on a diet containing 6.4 g of threonine per kg DM had higher (P<0.05) growth rates than those on diets containing 7.5, 8.0, 8.5 or 9.0 g per kg DM. However, chickens on diets containing 7.5, 8.0, 8.5 or 9.0 g per kg DM had similar (P>0.05) growth rates.

Dietary threonine level had no effect (P>0.05) on feed conversion ratio during Weeks 1 and 3 (Table 4.2). However, dietary threonine level affected feed conversion ratios of the chickens during Week 2. Unsexed Ross 308 broiler chickens on diets containing 7.5 or 9.0 g of threonine per kg DM had better (P<0.05) feed conversion ratio (FCR) than those on a diet containing 6.4 g of threonine per kg DM. However, broiler chickens on diets having 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) feed conversion ratios. Similarly, chickens on diets containing 6.4, 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) feed conversion ratios. Dietary threonine level affected (P>0.05) live weights of unsexed Ross 308 broiler chickens aged 7, 14 or 21 days (Table 4.2). Chickens aged 7 days and, on a diet, containing 6.4 g of threonine per kg DM had higher (P<0.05) live weights than those on diets containing 8.0 or 8.5 g of threonine per kg DM. However, chickens aged 7 days and on diets containing 6.4, 7.5 or 9.0 g of threonine per kg DM had similar (P>0.05) live weights. Similarly, chickens on diets containing 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) live weights. Unsexed Ross 308 broiler chickens aged 14 and 21 days and, on a diet, containing 6.4 g of threonine per kg DM had higher (P<0.05) live weights than those on diets containing 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM. However, chickens on diets containing 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had similar (P>0.05) live weights.

Negative relationships were observed between dietary threonine level and feed intake (r = 0.76), growth rate (r = 0.80) and live weight (r = 0.81) of the chickens aged 8 to 14 days (Figures 4.01, 4.02 and 4.04, respectively and Table 4.3). Feed conversion ratio was optimized (r^2 = 0.590) at dietary threonine level of 9.57 g per kg DM for broiler chickens aged 8 to 14 days (Figure 4.04 and Table 4.3).

Table 4.2 Effect of dietary threonine level on diet DM intake, digestibility, growth rate, live weight, feed conversion ratio, metabolisable energy and nitrogen retention of unsexed Ross 308 broiler chickens aged 1 to 21 days*

	Treatment #				
Variable	$UT_{6.4}$	$UT_{7.5}$	$UT_{8.0}$	$UT_{8.5}$	$UT_{9.0}$
Intake (g DM/chicken/day)					
Week 1	11^{a}±1.80	8^{ab}±0.33	7^{ab}±0.86	6^{b}±1.02	9^{ab}±1.16
Week 2	34^{a}±1.35	19^{ab}±0.85	18^{bc}±1.72	14^{c}±1.4	21^{b}±0.82

Week 3	39^{a}±2.23	36^{b}±0.83	36^{b}±2.77	33^{b}±1.29	40^{a}±1.78
Digestibility (%), Week 3					
DM	77^{a}±0.7	77^{a}±1.1	81^{a}±0.5	76^{a}±0.3	81^{a}±3.4
CP	28^{a}±5.0	39^{a}±8.7	41^{a}±16.1	51^{a}±6.0	54^{a}±16.0
NDF	19^{a}±4.3	24^{a}±2.2	19^{a}±0.2	17^{a}±2.7	12^{a}±4.1
ADF	42^{a}±3.1	35^{a}±0.9	30^{a}±1.1	55^{a}±5.9	57^{a}±5.9
Fat	60^{a}±1.0	63^{a}±5.1	65^{a}±11.9	67^{a}±3.3	55^{a}±9.6
Ash	29^{a}±7.0	21^{a}±2.4	37^{a}±2.1	13^{a}±5.3	39^{a}±4.1
ME intake (MJ/kg DM) Wk3	12.5^{b}±0.20	12.5^{b}±0.20	13.3^{a}±0.12	12.4^{c}±0.18	13.3^{a}±0.12
N-retn (g/bird/day) Wk3	1.9^{ab}±0.31	1.3^{bc}±0.10	2.0^{a}±0.07	1.2^{c}±0.15	2.4^{a}±0.30
Growth rate (g/chicken/day)					
Week 1	10^{a}±1.5	6^{a}±0.1	6^{a}±0.6	5^{a}±0.7	7^{a}±0.7
Week 2	21^{a}±0.6	8^{b}±0.4	8^{b}±0.9	7^{b}±0.8	9^{b}±0.7
Week 3	26^{a}±2.0	21^{a}±1.2	21^{a}±2.8	22^{a}±0.5	26^{a}±1.6
FCR (g DM feed intake/g weight gain)					
Week 1	1.2^{a}±0.01	1.2^{a}±0.03	1.2^{a}±0.07	1.2^{a}±0.04	1.3^{a}±0.08
Week 2	1.7^{b}±0.08	2.3^{a}±0.03	2.2^{ab}±0.06	2.0^{ab}±0.06	2.5^{a}±0.22
Week 3	1.9^{a}±0.07	1.8^{a}±0.06	1.7^{a}±0.09	1.5^{a}±0.06	1.6^{a}±0.03
Live weight (g/chicken)					
Day 7	111^{a}±10.3	88^{ab}±1.6	83^{b}±4.2	77^{b}±5.0	92^{ab}±5.0
Day 14	255^{a}±9.0	146^{b}±2.9	143^{b}±10.7	127^{b}±10.0	153^{b}±8.0
Day 21	436^{a}±16.2	290^{b}±11.0	291^{b}±30.0	298^{b}±11.4	332^{b}±18.3

* : 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)

: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

N-retn : Nitrogen retention

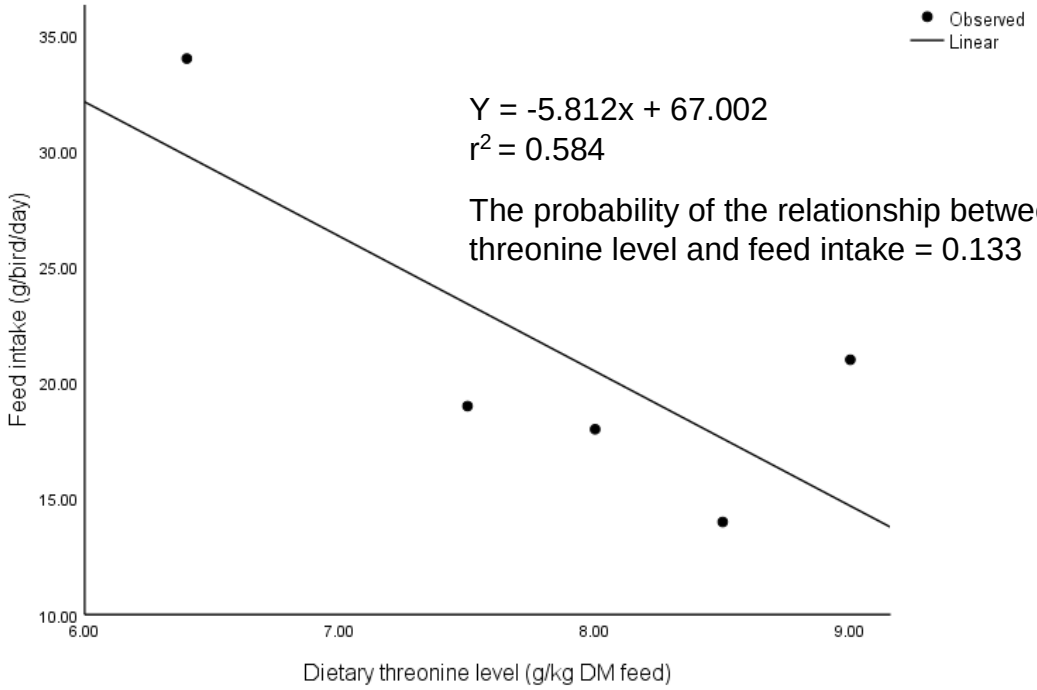

Figure 4.01 Relationship between dietary threonine level and feed intake of unsexed Ross 308 broiler chickens aged 8 to 14 days

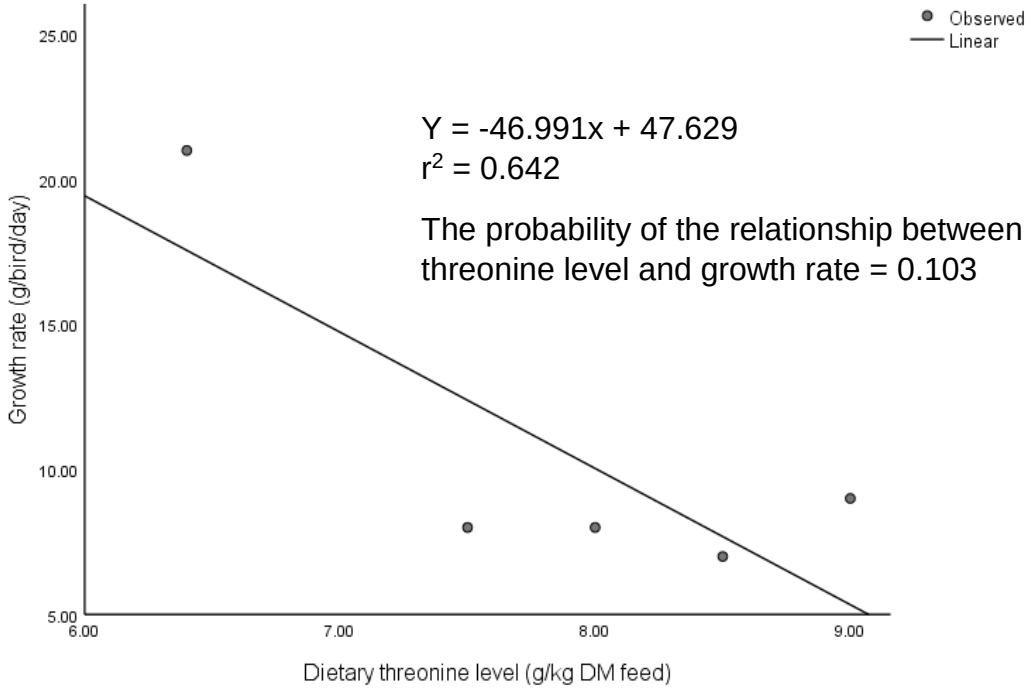

Figure 4.02 Relationship between dietary threonine level and growth rate of unsexed Ross 308 broiler chickens aged 8 to 14 days

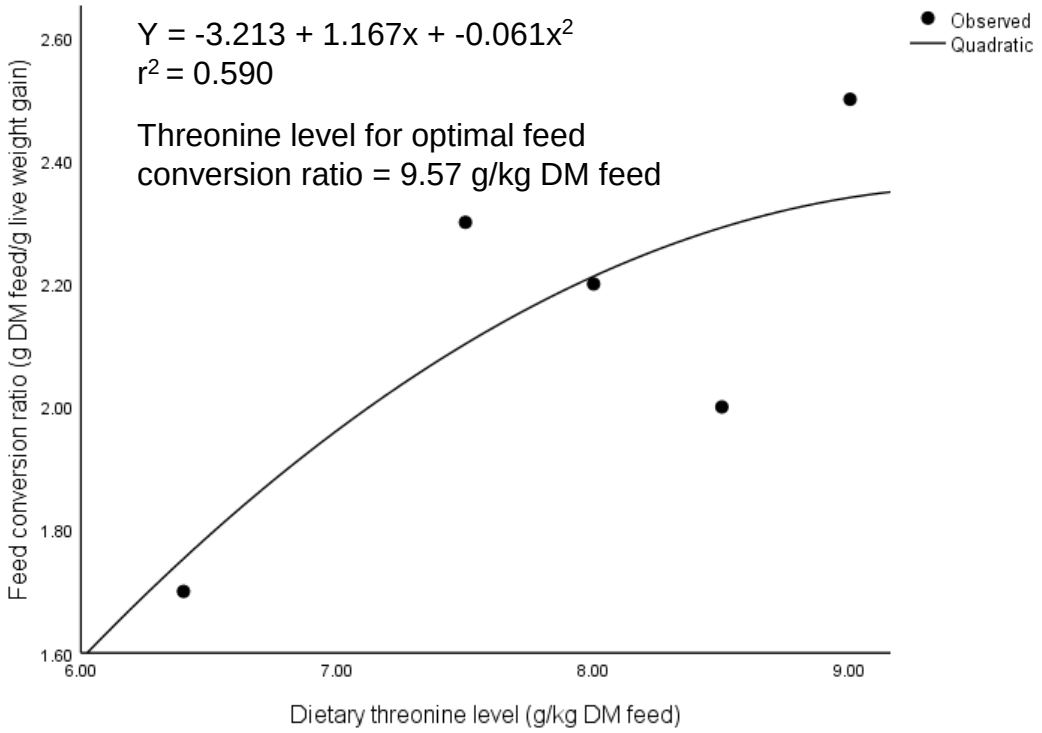

Figure 4.03 Effect of dietary threonine level on feed conversion ratio of unsexed Ross 308 broiler chickens aged 8 to 14 days

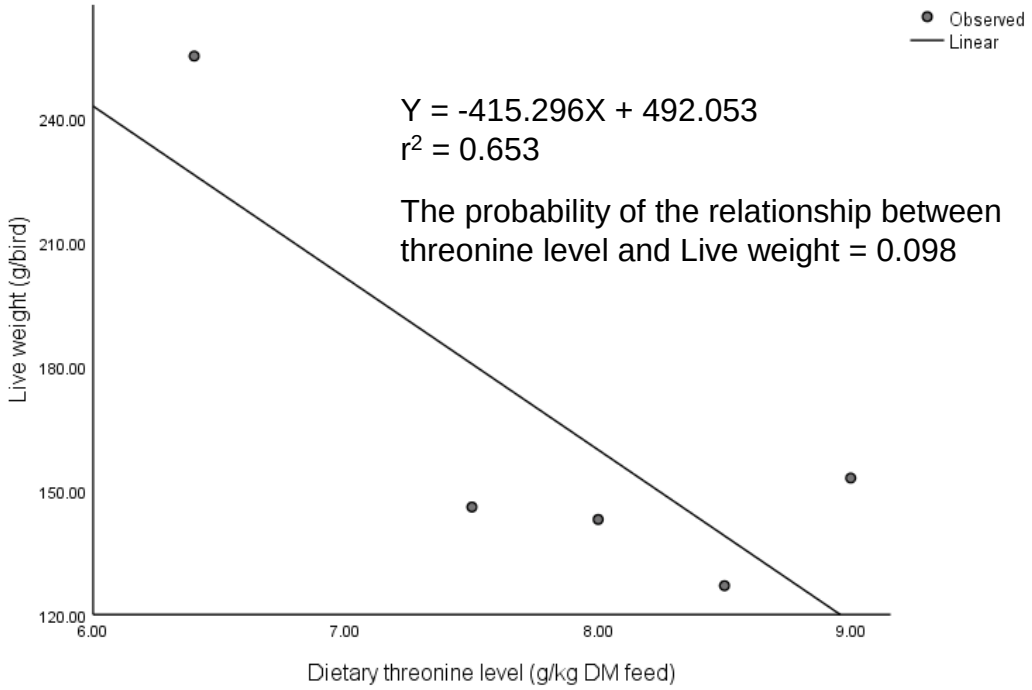

Figure 4.04 Relationship between dietary threonine level and live weight of unsexed Ross 308 broiler chickens aged 14 days

Table 4.3 Relationships between dietary threonine level and feed intake (aged 8 to 14 days) growth rate (aged 8 to 14 days) and live weight (aged 14 days) of unsexed Ross 308 broiler chickens

Variable	Formula	r^2	Probability
Feed intake (g DM/bird/day)	Y = -5.812x + 67.002	0.584	0.133
Growth rate (g/bird/day)	Y = -46.991X + 47.629	0.642	0.103
Live weight at (g/bird)	Y = -415.296x + 492.053	0.653	0.098

r^2 : Coefficient of determination

Results of the effects of threonine level on gut organ digesta pH, gut organ lengths and weights of male Ross 308 broiler chickens aged 21 days are presented in Table 4.4. Dietary threonine level had no effect (P>0.05) on gut organ digesta pH of crop, small and large intestine values of unsexed Ross 308 broiler chickens aged 21 days. However, dietary threonine level affected (P<0.05) digesta pH values of proventriculus, caecum and gizzards of the chickens aged 21 days. Broiler chickens on diets containing 7.5 or 9.0 g of threonine per kg DM had higher (P<0.05) proventriculus digesta pH values than those on a diet having 6.4 g of threonine per kg DM. However, broiler chickens on diets containing 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) proventriculus pH values. Similarly, broiler chickens on diets containing 6.4, 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) proventriculus digesta pH values. Unsexed broiler chickens on a diet containing 8.5 g of threonine per kg DM had higher (P<0.05) gizzard digesta pH values than those on diets having 6.4, 7.5, 8.0 or 9.0 g of threonine per kg DM. However, chicken on diets having 6.4, 7.5, 8.0 or 9.0 g of threonine per kg DM had the same (P>0.05) gizzard digesta pH values. Unsexed broiler chickens on a diet containing 7.5 g of threonine per kg DM had higher (P<0.05) caecum digesta pH values than those on diets having 8.0, 8.5 or 9.0 g of threonine per kg DM. However, chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had the same (P>0.05) caecum digesta pH values. Similarly, chickens on diets having 6.4, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) caecum digesta pH values.

Dietary threonine level had no effect (P>0.05) on small intestine lengths of unsexed Ross 308 broiler chickens aged 21 days (Table 4.4). However, dietary threonine level affected (P<0.05) gut intestine tract, caecum and large intestine lengths of the

chickens aged 21 days. Unsexed Ross 308 broiler chickens on diets having 6.4, 7.5 or 8.0 g of threonine per kg DM had higher ($P<0.05$) GIT lengths than those on diets having 8.5 or 9.0 g of threonine per kg DM. Chickens on a diet having 9.0 g of threonine per kg DM had higher ($P<0.05$) gut intestinal tract lengths than those on a diet containing 8.5 g of threonine per kg DM. Chickens on diets having 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had higher ($P<0.05$) caecum length values than those on a diet containing 6.4 g of threonine per kg DM. However, chickens on diets having 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same ($P>0.05$) caecum length values. Broiler chickens on a diet having 6.4 g of threonine per kg DM had higher ($P<0.05$) large intestine lengths than those on diets containing 8.0, 8.5 or 9.0 g of threonine per kg DM. Chickens on a diet having 7.5 g of threonine per kg DM had higher ($P<0.05$) large intestine lengths than those on a diet containing 8.0 g of threonine per kg DM. chickens on diets having 6.4 or 7.5 g of threonine per kg DM had the similar ($P>0.05$) large intestine lengths. Similarly, chickens on diets having 8.0, 8.5 or 9.0 g of threonine per kg DM had the same ($P>0.05$) large intestine lengths.

Dietary threonine level had no effect ($P>0.05$) on small intestine weights of unsexed Ross 308 broiler chickens aged 21 days (Table 4.4). However, dietary threonine level affected crop, proventriculus, gizzard, caecum and large intestine weights of the chickens aged 21 days. Unsexed Ross 308 broiler chickens on diets having 6.4 or 7.5 g of threonine per kg DM had higher ($P<0.05$) crop weights than those on diets containing 8.0, 8.5 or 9.0 g of threonine per kg DM. However, chickens on diets having 6.4 or 7.5 g of threonine per kg DM had similar ($P>0.05$) crop weights. Similarly, chickens on diets having 8.0, 8.5 or 9.0 g of threonine per kg DM had the same ($P>0.05$) crop weights. Chickens on a diet having 6.4 g of threonine per kg DM had higher ($P>0.05$) proventriculus weights than those on diets containing 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM. Similarly, chickens on diets having 8.0 or 9.0 g of threonine per kg DM had higher ($P<0.05$) proventriculus weights than those on diets containing 7.5 or 8.5 g of threonine per kg DM. However, chickens on diets having 8.0 or 9.0 g of threonine per kg DM had the same ($P>0.05$) proventriculus weights. Similarly, chickens on diets having 7.5 or 8.5 g of threonine per kg DM had the same ($P>0.05$) proventriculus weights. Unsexed broiler chickens on diets having 6.4 or 9.0 g of threonine per kg DM had higher ($P<0.05$) gizzard weights than those on diets containing 7.5, 8.0 or 8.5 g of threonine per kg DM. However, chickens on diets having

6.4 or 9.0 g of threonine per kg DM had the same (P>0.05) gizzard weights. Similarly, chickens on diets having 7.5, 8.0 or 8.5 g of threonine per kg DM had same (P>0.05) gizzard weights. Unsexed broiler chickens on a diet having 9.0 g of threonine per kg DM had higher (P<0.05) caecum weights than those on diets containing 6.4, 7.5, 8.0 or 8.5 g of threonine per kg DM. Chickens on diets having 7.5 or 8.5 g of threonine per kg DM had higher (P<0.05) gizzard weights than those on diets containing 6.4 or 8.0 g of threonine per kg DM. However, chickens on diets containing 7.5 or 8.5 g of threonine per kg DM had the same (P>0.05) gizzard weights. Similarly, chickens on diets having 6.4 or 8.5 g of threonine per kg DM had the same (P>0.05) gizzard weights. Broiler chickens on diets having 8.0 or 8.5 g of threonine per kg DM had higher (P<0.05) large intestine weights than those on diets containing 6.4, 7.5 or 9.0 g of threonine per kg DM. Chickens on a diet having 9.0 g of threonine per kg DM had higher (P<0.05) large intestine weights than those on a diet containing 6.4 g of threonine per kg DM. However, chickens on diets having 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) large intestine weights. Broiler chickens on diets having 7.5 or 9.0 g of threonine per kg DM had similar (P>0.05) large intestine weights. Similarly, chickens on diets having 6.4 or 7.5 g of threonine per kg DM had the same (P>0.05) large intestine weights.

Proventriculus digesta pH values of unsexed Ross 308 broiler chickens were optimized at a dietary threonine level of 8.45 (r = 0.92) g/kg DM (Figure 4.05). Gut intestinal tract lengths of unsexed Ross 308 broiler chickens aged 21 days were optimized at a dietary threonine level of 6.56 (r = 0.75) g per kg DM (Figure 4.06). Caecum length of unsexed Ross 308 broiler chickens aged 21 days was optimized at a dietary threonine level of 8.40 g (r = 0.90) per kg DM (Figure 4.07). A negative relationship was observed between dietary threonine level and large intestine lengths (r = 0.76) of chickens aged 21 days (Figure 4.08).

Table 4.4 Effect of dietary threonine level on digesta pH value, length and weight of gastrointestinal organs of unsexed Ross 308 broiler chickens aged 21 days*

	Treatment #				
Variable	$UT_{6.4}$	$UT_{7.5}$	$UT_{8.0}$	$UT_{8.5}$	$UT_{9.0}$
Gut organ digesta pH					
Crop	3.8^{a}±0.04	4.2^{a}±0.30	3.9^{a}±0.06	4.1^{a}±0.30	4.0^{a}±0.08
Proventriculus	3.9^{b}±0.10	4.4^{a}±0.20	4.3^{ab}±0.12	4.3^{ab}±0.12	4.4^{a}±0.20
Gizzard	1.7^{b}±0.07	1.8^{b}±0.02	1.9^{b}±0.10	2.2^{a}±0.08	1.8^{b}±0.02
Small intestines	5.7^{a}±0.17	5.9^{a}±0.38	5.7^{a}±0.10	5.8^{a}±0.08	5.7^{a}±0.05
Caecum	6.5^{ab}±0.19	7.1^{a}±0.08	5.9^{b}±0.13	6.1^{b}±0.10	6.2^{b}±0.13
Large intestines	4.4^{a}±0.20	4.4^{a}±0.01	5.0^{a}±0.14	4.7^{a}±0.04	4.6^{a}±0.20
Gut organ length (cm)					
GIT	152.8^{a}±1.30	155.3^{a}±0.72	151.0^{a}±1.44	134.0^{c}±2.60	142.3^{b}±1.91
Small intestine	134.0^{a}±0.01	137.3^{a}±0.43	131.8^{a}±1.59	134.4^{a}±4.27	88.2^{a}±39.37
Caecum	5.5^{b}±0.29	13.8^{a}±0.73	10.8^{a}±0.03	13.4^{a}±0.45	13.0^{a}±1.80
Large intestine	11.3^{a}±0.14	10.5^{ab}±0.29	8.7^{c}±0.43	9.7^{cb}±0.12	9.5^{cb}±0.29
Gut organ weight (g)					
Crop	4.5^{a}±0.03	4.6^{a}±0.14	2.9^{b}±0.20	2.9^{b}±0.26	3.2^{b}±0.14
Proventriculus	7.7^{a}±0.17	3.2^{c}±0.01	4.3^{b}±0.17	3.6^{c}±0.06	4.7^{b}±0.12
Gizzard	14.2^{a}±0.64	11.2^{b}±0.29	11.0^{b}±0.26	8.8^{b}±0.35	14.2^{a}±0.32
Small intestines	32.3^{a}±0.03	29.3^{a}±0.29	30.5^{a}±1.04	32.7^{a}±1.27	30.9^{a}±0.61
Caeca	0.9^{c}±0.03	1.3^{b}±0.09	0.7^{c}±0.03	1.3^{b}±0.03	1.8^{a}±0.03
Large intestines	1.6^{c}±0.09	1.7^{bc}±0.01	3.5^{a}±0.06	3.1^{a}±0.01	2.2^{b}±0.23

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

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

\# : Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

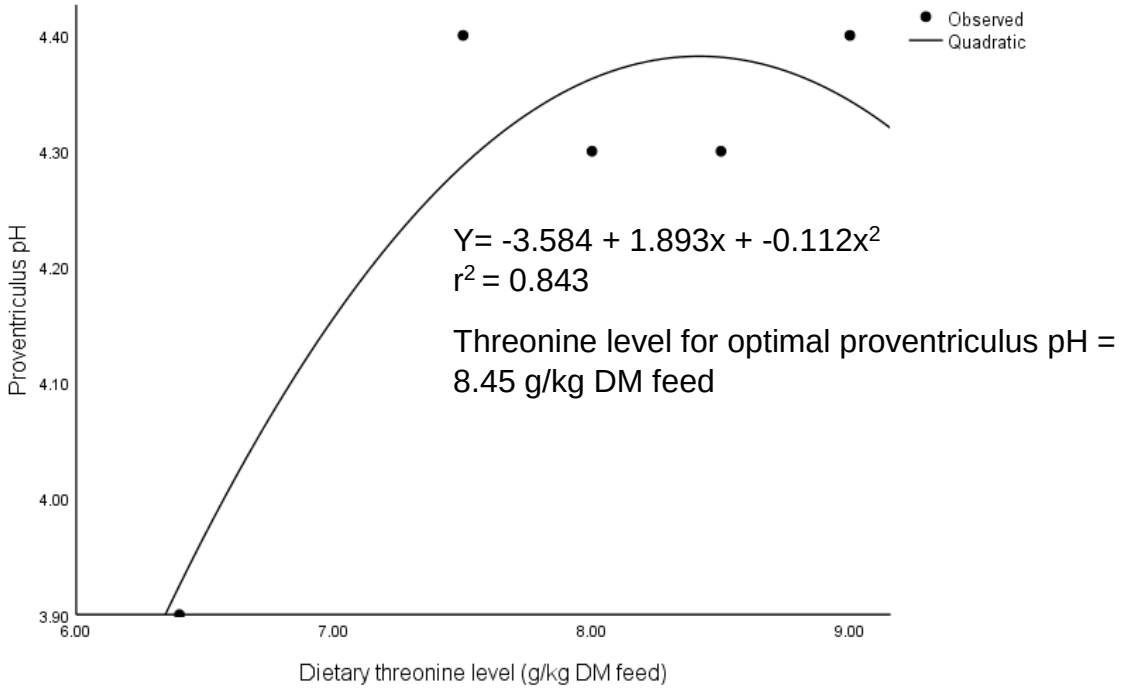

Figure 4.05 Effect of dietary threonine level on proventriculus digesta pH of unsexed Ross 308 broiler chickens aged 21 days

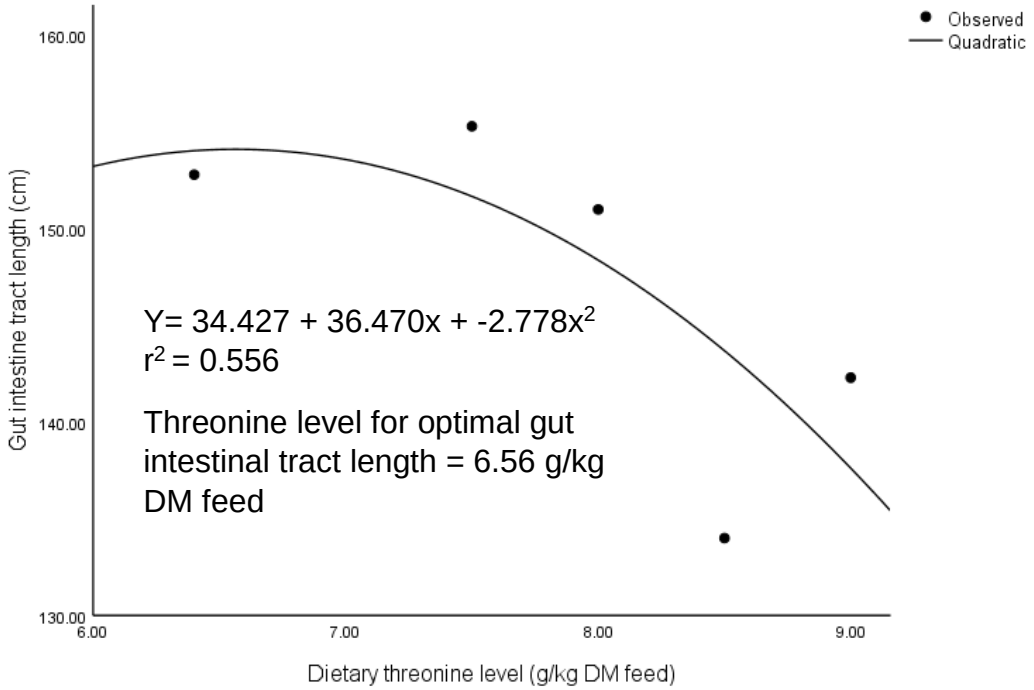

Figure 4.06 Effect of dietary threonine level on gut intestinal tract length of unsexed Ross 308 broiler chickens aged 21 days

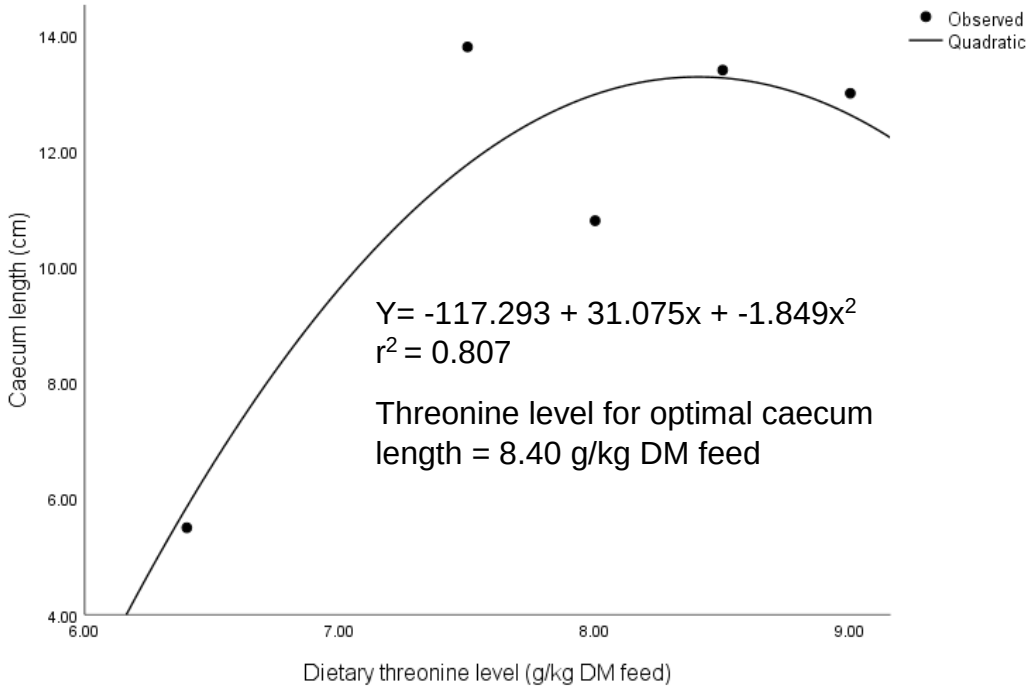

Figure 4.07 Effect of dietary threonine level on caecum lengths of unsexed Ross 308 broiler chickens aged 21 days

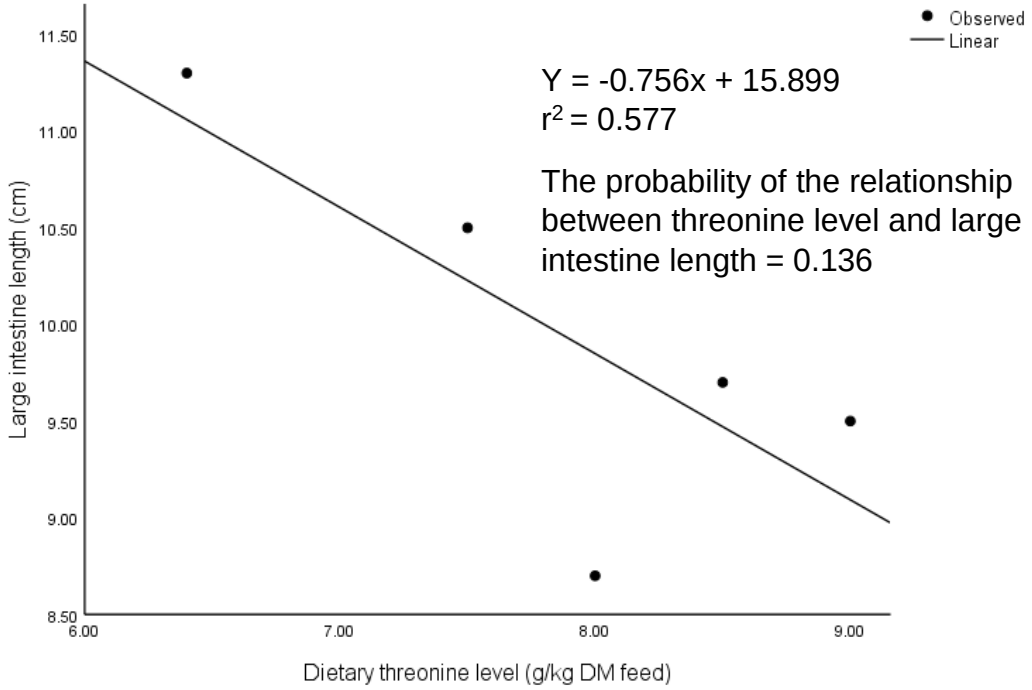

Figure 4.08 Relationship between dietary threonine level and large intestine lengths of unsexed Ross 308 broiler chickens aged 21 days

4.2 Broiler chickens aged 22 to 42 days

Results of the nutrient composition of the experimental diets are presented in Table 4.5. The experimental diets were isocaloric and isonitrogenous, but with different threonine levels. All the diets contained 20% crude protein and 17 MJ of energy per kg DM as recommended by NRC (1994) for broiler chickens aged 22 to 42 days.

Table 4.5 Diet composition (% except MJ/kg DM for energy and g/kg DM for threonine) for Experiment 2

	Treatment[#]				
Nutrient	$UT_{6.4}$	$UT_{7.5}$	$UT_{8.0}$	$UT_{8.5}$	$UT_{9.0}$
DM	90.34	90.34	90.34	90.34	90.34
CP	20	20	20	20	20
Energy	17	17	17	17	17
ADF (%)	5	5	4.63	5.38	5.81
NDF (%)	17.04	17.4	17	17.3	17.1
Fat (%)	4.51	4.95	5.37	5.46	5.23
Ash (%)	6.45	6.77	6.66	6.62	6.32
Ca (%)	1.01	1.02	1.01	1.00	1.01
Threonine	6.4	7.5	8	8.5	9

: Treatment codes are explained in Chapter 3, Table 3.03 (The treatments were dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

Results of the effects of threonine level on feed intake, digestibility, growth rate, live weight and feed conversion ratio of male Ross 308 broiler chickens aged 22 to 42 days are presented in Table 4.6. The dietary threonine level had no effect ($P>0.05$) on DM feed intakes of male Ross 308 broiler chickens during Weeks 4 and 6. However, dietary threonine level affected ($P<0.05$) feed intake of the chickens during Week 5. Male Ross 308 broiler chickens on diets containing 9.0 g of threonine per kg DM had higher ($P<0.05$) feed intakes than those on diets containing 6.4, 7.5 or 8.5 g of threonine per kg DM. However, chickens on diets containing 8.0 or 9.0 g of threonine per kg DM had similar ($P>0.05$) feed intakes. Similarly, chickens on diets containing 6.4, 7.5, 8.0 or 8.5 g of threonine per kg DM had the same ($P>0.05$) feed intakes. Dietary threonine level affected ($P<0.05$) diet DM, CP, NDF, ADF, fat and ash digestibility (Table 4.6). Dietary threonine level affected ($P<0.05$) diet DM digestibility of male Ross 308 broiler chickens for Week 5. Male Ross 308 broiler chickens on a diet containing 8.0 g of threonine per kg DM had higher ($P<0.05$) diet DM digestibility

values than those on diets containing 8.5 or 0.9 g of threonine kg DM. Similarly, male broiler chickens on diets containing 8.5 or 9.0 g of threonine per kg DM had higher ($P<0.05$) diet DM digestibility values than those on diets containing 6.4 or 7.5 g of threonine per kg DM. Male Ross 308 broiler chickens on diets containing 8.5 or 9.0 g of threonine per kg DM had the same ($P>0.05$) diet DM digestibility values. Similarly, chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had the same ($P>0.05$) diet DM digestibility values. Chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had higher ($P<0.05$) diet CP digestibility values than those on a diet containing 8.0 g of threonine per kg DM. However, male broiler chickens on diets containing 8.5 or 9.0 g of threonine per kg DM had the same ($P>0.05$) CP diet digestibility values. Male Ross 308 broiler chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had the same ($P>0.05$) diet CP digestibility values. Similarly, male broiler chickens on diets having 8.0 or 9.0 g of threonine per kg DM had the same ($P>0.05$) CP diet digestibility values.

Results of the present study indicate that dietary threonine level affected ($P<0.05$) diet NDF and ADF digestibility values (Table 4.6). Male Ross 308 broiler chickens on a diet containing 8.0 g of threonine per kg DM had higher ($P<0.05$) diet NDF digestibility values than those on diets having 6.4, 7.5 or 8.5 g of threonine per kg DM. However, male broiler chickens on 8.0 or 9.0 g of threonine per kg DM had similar ($P>0.05$) diet NDF digestibility values. Similarly, broiler chickens on diets having 6.4, 7.5, 8.5 or 9.0 g of threonine per kg DM had the same ($P>0.05$) diet NDF digestibility values. Male broiler chickens on a diet having 8.0 g of threonine per kg DM had higher ($P<0.05$) diet ADF digestibility values than those on a diet having 9.0 g of threonine per kg DM. Similarly, chickens on a diet having 9.0 g of threonine per kg DM had higher ($P<0.05$) diet ADF digestibility values than those on diets containing 6.4 or 7.5 g of threonine per kg DM. Similarly, chickens on a diet having 6.4 g of threonine per kg DM had higher ($P<0.05$) diet ADF digestibility values than those on a diet containing 7.5 g of threonine per kg DM. Broiler chickens on diets having 8.0 or 8.5 g of threonine per kg DM had the same ($P>0.05$) diet ADF digestibility values. Similarly, chickens on diets having 9.0 or 8.5 g of threonine per kg DM had the same ($P>0.05$) diet ADF digestibility values. Dietary threonine level of male Ross 308 broiler chickens aged 22 to 42 days affected ($P<0.05$) diet ash digestibility (Table 4.6). Male broiler chickens on diets having 8.0 or 9.0 g of threonine per kg DM had higher ($P<0.05$) diet ash digestibility

values than those on diets containing 6.4 or 7.5 g of threonine per kg DM. However, broiler chickens on diets having 8.0, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) diet ash digestibility values. Similarly, male broiler chickens on diets having 6.4, 7.5 or 8.5 g of threonine per kg DM had the same (P>0.05) ash digestibility values.

Dietary threonine level had no effect (P>0.05) on nitrogen retention values of male Ross 308 broiler chickens aged 36 to 42 days (Table 4.6). However, dietary threonine level affected (P<0.05) metabolisable energy intakes of male broiler chickens. Male Ross 308 broiler chickens on a diet containing 8.0 g of threonine per kg DM had higher (P<0.05) metabolisable energy intakes than those on diets containing 6.4, 7.5, 8.5 or 9.0 g of threonine per kg DM. Chickens on a diet containing 9.0 g of threonine per kg DM had higher (P<0.05) metabolisable energy intakes than those on diets containing 6.4, 7.5, or 8.5 g per kg DM. Similarly, chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had higher (P<0.05) metabolisable energy intakes than those on a diet containing 8.5 g per kg DM. However, broiler chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had similar (P>0.05) metabolisable energy intakes.

Dietary threonine level had no effect (P>0.05) on the growth rates and feed conversion ratios of male Ross 308 broiler chickens aged 22 to 42 days (Table 4.6). Results of the present study indicate that dietary threonine level had no effect (P>0.05) on the live weights of male Ross 308 broiler chickens aged 35 or 42 days. However, dietary threonine level affected (P<0.05) live weights of male Ross 308 broiler chickens aged 28 days. Male Ross 308 broiler chickens on a diet containing 6.4 g of threonine per kg DM had higher (P<0.05) live weights than those on a diet containing 7.5 g per kg DM. However, male Ross 308 broiler chickens on diets containing 6.4, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) live weights. Similarly, male Ross 308 broiler chickens on diets containing 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had similar (P>0.05) live weights.

A positive relationship was observed between dietary threonine level and feed intake ($r^2 = 0.89$) of male Ross 308 broiler chickens aged 28 to 35 days (Figure 4.9).

Table 4.6 Effect of dietary threonine level on diet DM intake, digestibility, growth rate, live weight. feed conversion ratio, metabolisable energy intake and nitrogen retention of male Ross 308 broiler chickens aged 22 to 42 days*

	Treatment #				
Variables	$MT_{6.4}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Intake (g/chicken/day)					
Week 4	90^{a}±2.1	82^{a}±2.2	82^{a}±2.2	84^{a}±7.3	104^{a}±10.0
Week 5	78^{b}±3.1	78^{b}±3	95^{ab}±15.0	93^{b}±0.2	101^{a}±6.7
Week 6	132^{a}±6.8	112^{a}±2.7	140^{a}±9.9	139^{a}±6.3	144^{a}±15.8
Digestibility (%), Week 6					
DM	76^{c}±0.3	76^{c}±0.7	89^{a}±1.7	81^{b}±0.1	83^{b}±0.7
CP	27^{a}±5.2	41^{a}±8.3	3^{c}±1.0	11^{b}±3.3	6^{bc}±2.5
NDF	27^{b}±2.4	26^{b}±4.0	53^{a}±0.2	32^{b}±2.1	38^{ab}±3.3
ADF	40^{c}±2.4	22^{d}±12.0	68^{a}±0.6	58^{ab}±0.8	58^{b}±2.4
Ash	19^{b}±5.0	18^{b}±8.3	76^{a}±12.0	51^{ab}±1.7	70^{a}±7.4
ME (MJ/kg DM) Wk6	13.2^{d}±0.10	13.2^{d}±0.10	15.4^{a}±0.19	14.1^{c}±0.06	14.4^{b}±0.18
N-rent (g/bird/day) Wk6	2.6^{a}±0.46	3.9^{a}±1.29	3.2^{a}±0.21	2.6^{a}±0.29	3.3^{a}±0.47
Growth rate (g/chicken/day)					
Week 4	33^{a}±9.1	24^{a}±4.4	42^{a}±4.0	39^{a}±5.0	44^{a}±3.5
Week 5	43^{a}±9.9	44^{a}±2.5	52^{a}±17.5	69^{a}±3.0	59^{a}±11.9
Week 6	75^{a}±12.9	59^{a}±3.2	97^{a}±30.4	66^{a}±2.5	76^{a}±15.4
FCR (g feed intake/g weight gain)					
Week 4	3.1^{a}±0.71	3.6^{a}±0.58	2.0^{a}±0.14	2.2^{a}±0.22	2.4^{a}±0.15
Week 5	1.9^{a}±0.20	1.8^{a}±0.05	2.3^{a}±0.74	1.4^{a}±0.06	1.83^{a}±0.32
Week 6	1.8^{a}±0.20	1.9^{a}±0.10	1.7^{a}±0.43	2.1^{a}±0.06	2.0^{a}±0.22
Live weight (g/chicken)					
Day 28	668^{a}±60.0	460^{b}±21.1	586^{ab}±41.1	549^{ab}±43.7	638^{ab}±31.5
Day 35	968^{a}±127.0	767^{a}±36.7	951^{a}±155.5	1033^{a}±23.1	1052^{a}±92.6
Day 42	1496^{a}±135.0	1180^{a}±29.3	1629^{a}±140	1491^{a}±27.7	1581^{a}±200.0

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

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

\# : Treatment codes are explained in Chapter 3, Table 3.03 (The treatments were dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

N-retn : Nitrogen retention

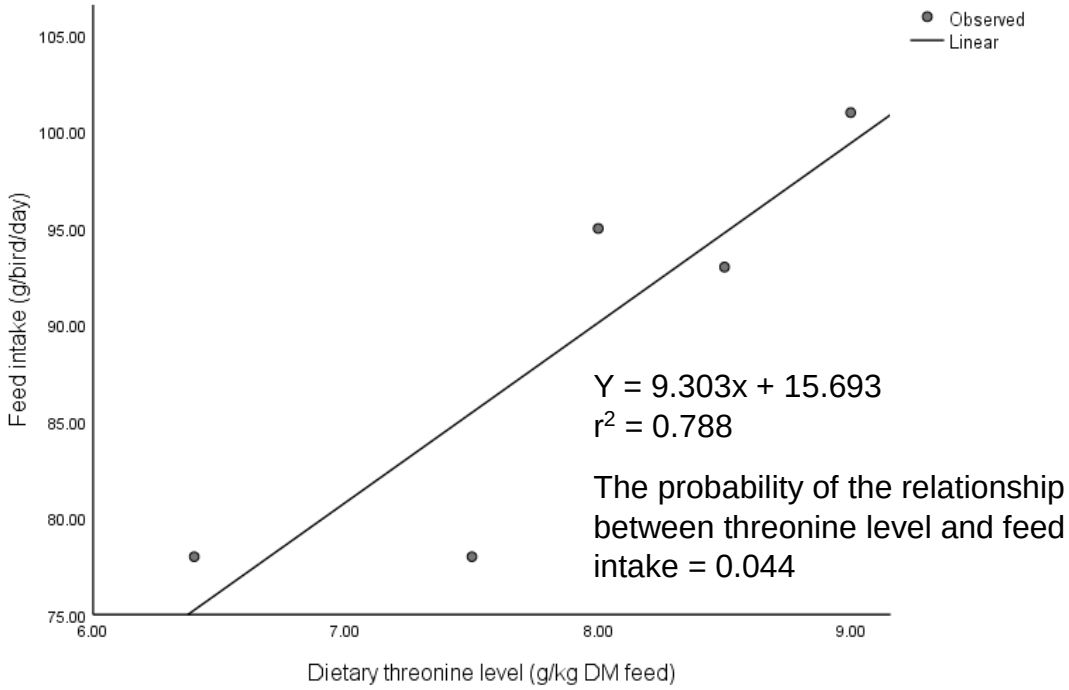

Figure 4.09 Relationship between dietary threonine level and feed intake of male Ross 308 broiler chickens aged 28 to 35 days

Results of the effects of dietary threonine level on gut organ digesta pH values, gut organ lengths and weights of male Ross 308 broiler chickens aged 42 days are presented in Table 4.7. Dietary threonine level had no effect (P>0.05) on gut organ digesta pH values of crop, proventriculus, caecum, small and large intestines of unsexed Ross 308 broiler chickens aged 42 days. However, dietary threonine level affected (P<0.05) gizzard digesta pH values of male Ross 308 broiler chickens aged 42 days. Broiler chickens on a diet containing 9.0 g of threonine per kg DM had higher (P<0.05) gizzard digesta pH values than those on a diet having 6.4 g of threonine per kg DM. However, male Ross 308 broiler chickens on diets containing 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had similar (P>0.05) gizzard digesta pH values. Similarly, broiler chickens on diets containing 6.4, 7.5, 8.0, or 8.5 g of threonine per kg DM had the same (P>0.05) gizzard digesta pH values.

Dietary threonine level had no effect (P>0.05) on caecum, small and large intestine lengths of male Ross 308 broiler chickens aged 42 days. However, dietary threonine level affected (P<0.05) gut intestinal tract lengths of the chickens aged 42 days. Chickens on diets having 6.4, 8.0, 8.5 or 9.0 g of threonine per kg DM had higher (P<0.05) GIT lengths than those on a diet having 7.5 g of threonine per kg DM. Male

broiler chickens on diets having 6.4, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) GIT length values.

Dietary threonine level affected (P<0.05) crop, proventriculus, gizzard, small intestine, caecum and large intestine weights of male Ross 308 broiler chickens aged 42 days (Table 4.7). Male Ross 308 broiler chickens on diets having 8.5 or 9.0 g of threonine per kg DM had higher (P<0.05) crop weights than those on diets containing 6.4, 7.5 or 8.0 g of threonine per kg DM. However, chickens on diets having 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) crop weights. Similarly, chickens on diets having 6.4, 7.5 or 8.0 g of threonine per kg DM had the same (P>0.05) crop weights. Male broiler chickens on a diet having 8.0 g of threonine per kg DM had higher (P>0.05) proventriculus weights than those on a diet containing 9.0 g of threonine per kg DM. However, chickens on diets having 6.4, 7.5, 8.0 or 8.5 g of threonine per kg DM had similar (P>0.05) proventriculus weights. Similarly, chickens on diets having 6.4, 7.5, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) proventriculus weights.

Broiler chickens on a diet having 9.0 g of threonine per kg DM had higher (P<0.05) gizzard weights than those on diets containing 6.4, 7.5, 8.0 or 8.5 g of threonine per kg DM (Table 4.7). Chickens on a diet having 6.4 g of threonine per kg DM had higher (P>0.05) gizzard weights than those on a diet containing 7.5 g of threonine per kg DM. Male broiler chickens on diets having 6.4 or 9.0 g of threonine per kg DM had similar (P>0.05) gizzard weights. Similarly, chickens on diets having 6.4, 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) gizzard weights. Chickens on diets having 7.5, 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) gizzard weights. Male broiler chickens on diets having 8.5 or 9.0 g of threonine per kg DM had higher (P<0.05) small intestine weights than those on diets containing 6.4, 7.5, or 8.0 g of threonine per kg DM. Similarly, broiler chickens on diets having 7.5 or 8.0 g of threonine per kg DM had higher (P<0.05) small intestine weights than those on a diet containing 6.4 g of threonine per kg DM. However, male broiler chickens on diets having 8.5 or 9.0 g of threonine per kg DM had similar (P<0.05) small intestine weights. Similarly, broiler chickens on diets having 7.5 or 8.0 g of threonine per kg DM had the same (P<0.05) small intestine weights.

Male broiler chickens on a diet having 9.0 g of threonine per kg DM had higher ($P<0.05$) caecum weights than those on diets containing 6.4, 8.0 or 8.5 g of threonine per kg DM (Table 4.7). Chickens on a diet having 7.5 g of threonine per kg DM had higher ($P<0.05$) caecum weights than those on a diet containing 8.5 g of threonine per kg DM. However, chickens on diets having 9.0 or 7.5 g of threonine per kg DM had the same ($P<0.05$) caecum weights. Broiler chickens on diets having 6.4, 7.5 or 8.0 g of threonine per kg DM had the same ($P>0.05$) caecum weights. Similarly, chickens on diets having 6.4, 8.0 or 8.5 had the same ($P>0.05$) caecum weights. Broiler chickens on a diet having 9.0 g of threonine per kg DM had higher ($P<0.05$) large intestine weights than those on a diet containing 6.4 g of threonine per kg DM. However, chickens on diets having 7.5, 8.0, 8.5 or 9.0 g of threonine per kg DM had the same ($P>0.05$) large intestine weights. Similarly, chickens on diets having 6.4, 7.5, 8.0 or 8.5 g of threonine per kg DM had the same ($P>0.05$) large intestine weights.

A positive relationship was observed between dietary threonine level and small intestine weights ($r = 0.98$) of male Ross 308 broiler chickens aged 42 days (Figure 4.11). Proventriculus and large intestine weights of male Ross 308 broiler chickens were optimized at dietary threonine levels of 7.49 ($r = 0.79$) and 9.07 ($r = 0.99$) g/kg DM, respectively (Figures 4.10 and 4.12).

Table 4.7 Effect of dietary threonine level on gut organ digesta pH, length and weight of male Ross 308 broiler chickens aged 42 days*

	Treatment #				
Variable	$MT_{6.4}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Gut organ digesta pH					
Crop	4.3^{a}±0.10	4.1^{a}±0.23	3.8^{a}±0.4	4.0^{a}±0.08	4.0^{a}±0.11
Proventriculus	3.6^{a}±0.04	4.0^{a}±0.18	3.5^{a}±0.18	3.5^{a}±0.12	3.7^{a}±0.22
Gizzard	2.3^{b}±0.14	2.6^{ab}±0.08	3.0^{ab}±0.30	3.1^{ab}±0.07	3.2^{a}±0.09
Small intestines	5.9^{a}±0.03	5.5^{a}±0.18	5.3^{a}±0.09	5.1^{a}±0.17	5.4^{a}±0.22
Caecum	6.3^{a}±0.23	6.0^{a}±0.08	6.0^{a}±0.16	6.2^{a}±0.20	5.9^{a}±0.10
Large intestines	5.7^{a}±0.13	5.6^{a}±0.12	5.6^{a}±0.18	5.6^{a}±0.18	5.7^{a}±0.15
Gut organ length (cm)					
GIT	206^{a}±2.6	182^{b}±1.6	211^{a}±4.1	212^{a}±2.1	213^{a}±1.8
Small intestines	182^{a}±3.7	162^{a}±7.1	177^{a}±10.4	186^{a}±5.4	178^{a}±7.0
Caecum	18^{a}±1.0	17^{a}±0.9	19^{a}±0.9	20^{a}±0.7	18^{a}±0.5
Large intestines	12^{a}±0.3	10^{a}±0.8	12^{a}±0.5	13^{a}±0.5	12^{a}±0.7
Gut organ weight (g)					
Crop	7.6^{b}±0.19	6.5^{b}±1.5	7.2^{b}±0.34	10.0^{a}±0.14	9.6^{a}±0.58
Proventriculus	10.1^{ab}±0.03	10.1^{ab}±0.40	10.8^{a}±0.25	10.2^{ab}±0.15	9.4^{b}±0.28
Gizzard	35.7^{ab}±1.53	30.1^{c}±0.74	32.1^{bc}±0.78	34.2^{bc}±1.00	41.0^{a}±1.60
Small intestines	64.9^{c}±3.71	77.5^{b}±0.78	83.9^{b}±1.87	98.1^{a}±1.83	100.2^{a}±2.94
Caecum	5.7^{bc}±0.45	6.8^{ab}±0.42	5.7^{bc}±0.10	5.3^{c}±0.10	7.3^{a}±0.09
Large intestines	4.9^{b}±0.16	6.2^{ab}±0.72	6.6^{ab}±0.09	6.6^{ab}±0.44	6.9^{a}±0.28

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

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

: Treatment codes are explained in Chapter 3, Table 3.03 (The treatments we dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

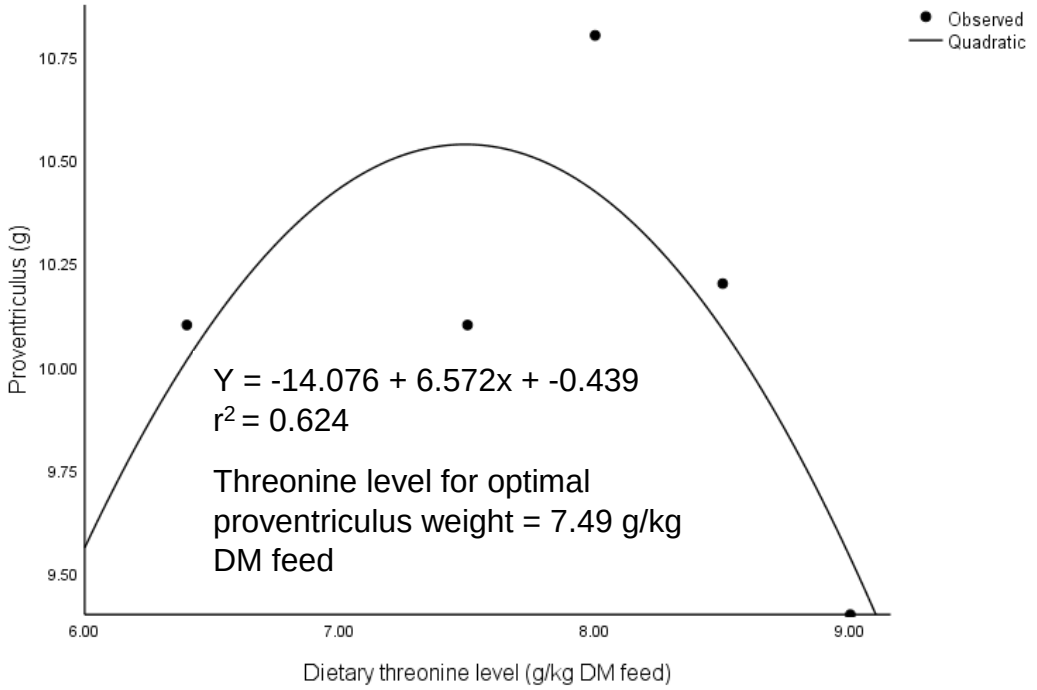

Figure 4.10 Effect of dietary threonine level on proventriculus weights of male Ross 308 broiler chickens aged 42 days

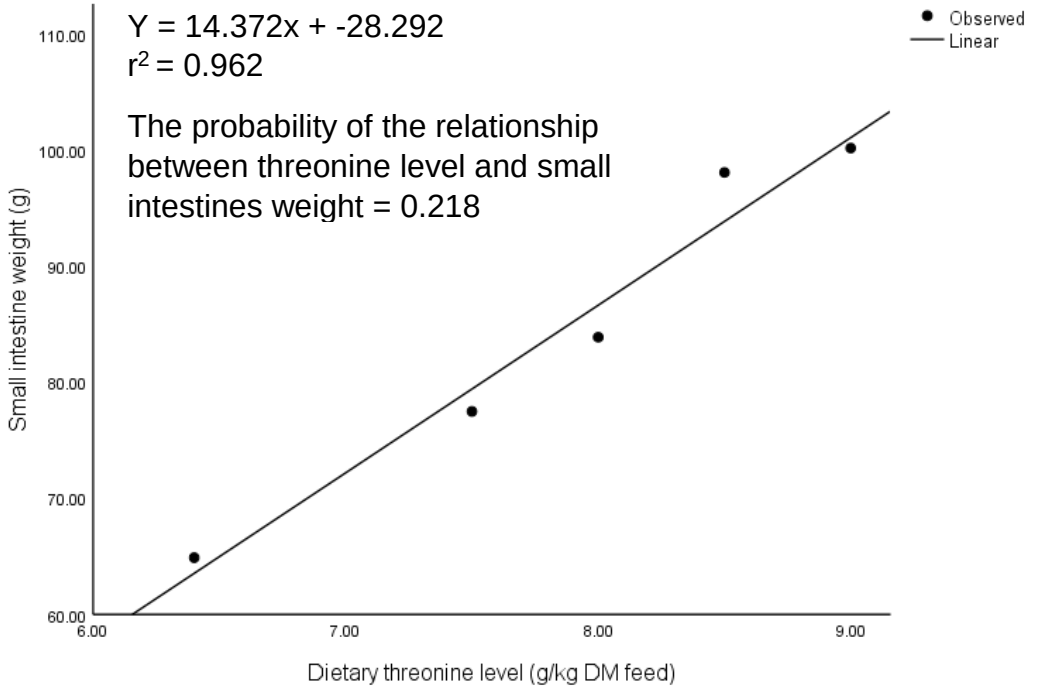

Figure 4.11 Relationship between dietary threonine level and small intestine weights of male Ross 308 broiler chickens aged 42 days

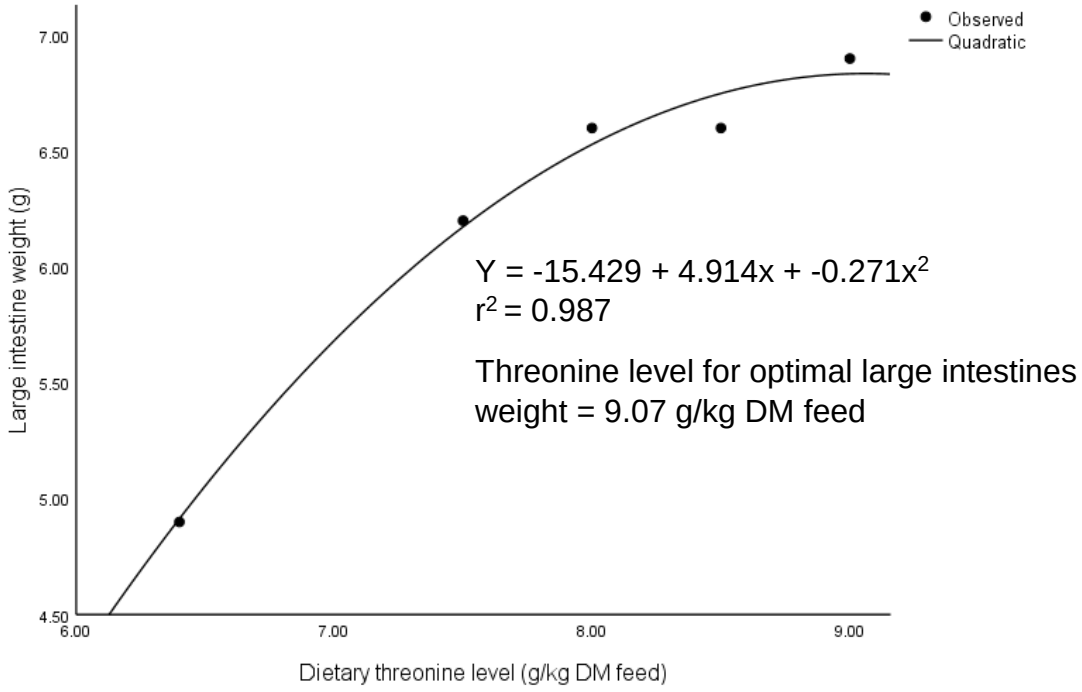

Figure 4.12 Effect of dietary threonine level on large intestine weights of male Ross 308 broiler chickens aged 42 days

Results on the effects of dietary threonine level on carcass weights of male Ross 308 broiler chickens aged 42 days are presented in Table 4.8. The study indicates that dietary threonine level had effect (P<0.05) on breast, carcass, drumstick and thigh weights of male Ross 308 broiler chickens aged 42 days. Male Ross 308 broiler chickens on a diet containing 9.0 g of threonine per kg DM had heavier (P<0.05) carcass weights than those on diets containing 6.4, 7.5, 8.0 or 8.5 g of threonine per kg DM. Male Ross 308 broiler chickens on diets containing 8.0 or 8.5 g of threonine per kg DM had heavier (P<0.05) carcass weights than those on a diet containing 7.5 g of threonine per kg DM. However, chickens on diets containing 6.4, 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) carcass weights. Similarly, chickens on diets containing 6.4 or 7.5 g of threonine per kg DM had the same (P>0.05) carcass weights. Male Ross 308 broiler chickens on a diet containing 9.0 g of threonine per kg DM had heavier (P<0.05) breast meat weights than those on diets containing 6.4 or 7.5 g of threonine per kg DM. However, male Ross 308 broiler chickens on diets containing 8.0, 8.5 or 9.0 g of threonine per kg DM had similar (P>0.05) breast meat weights. Similarly, chickens on diets containing 6.4, 7.5, 8.0 or 8.5 g of threonine per kg DM had the same (P>0.05) breast meat weights. Male Ross 308 broiler chickens

on a diet containing 9.0 g of threonine per kg DM had heavier (P<0.05) drumstick weights than those on diets containing 6.4 or 8.0 g of threonine per kg DM. Similarly, male Ross 308 broiler chickens on diets containing 6.4 or 8.0 g of threonine per kg DM had heavier (P<0.05) drumstick weights than on a diet containing 7.5 g of threonine per kg DM. However, chickens on diets containing 6.4, 8.0 or 8.5 g of threonine per kg DM had the similar (P>0.05) drumstick weights. Similarly, chickens on diets containing 7.5 or 8.5 g of threonine per kg DM had same (P>0.05) drumstick weights. Male Ross 308 broiler chickens on a diet containing 8.0 g of threonine per kg DM had heavier (P<0.05) thigh weights than those on a diet containing 8.5 g of threonine per kg DM. Chickens on a diet containing 8.5 g of threonine per kg DM had heavier (P<0.05) thigh weights than those on a diet having 7.5 g of threonine per kg DM. However, male Ross 308 broiler chickens on diets containing 6.4, 8.0 or 9.0 g of threonine per kg DM had similar (P>0.05) thigh weights. Similarly, male chickens on diets containing 6.4, 8.5 or 9.0 g of threonine per kg DM had the same (P>0.05) thigh weights.

A positive relationship was observed between dietary threonine level breast weights (r = 0.81) of male Ross 308 broiler chickens aged 42 days (Figure 4.13).

Table 4.8 Effect of dietary threonine level on carcass organ weights (g) of male Ross 308 broiler chickens aged 42 days*

	Treatment #				
Variable	$MT_{6.4}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Carcass	1126^{bc}±93.3	940^{c}±29.8	1169^{b}±44	1171^{b}±3.9	1407^{a}±3.2
Breast	326^{b}±21.7	297^{b}±21.5	382^{ab}±21.8	382^{ab}±21.7	471^{a}±7.0
Drumstick	166^{b}±4.6	138^{c}±3.5	167^{b}±5.1	149^{bc}±0.8	201^{a}±5.2
Thigh	193^{ab}±5.2	126^{c}±2.1	203^{a}±1.9	174^{b}±1.2	187^{ab}±8.3

* : 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)

: Treatment codes are explained in Chapter 3, Table 3.03 (The treatments dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

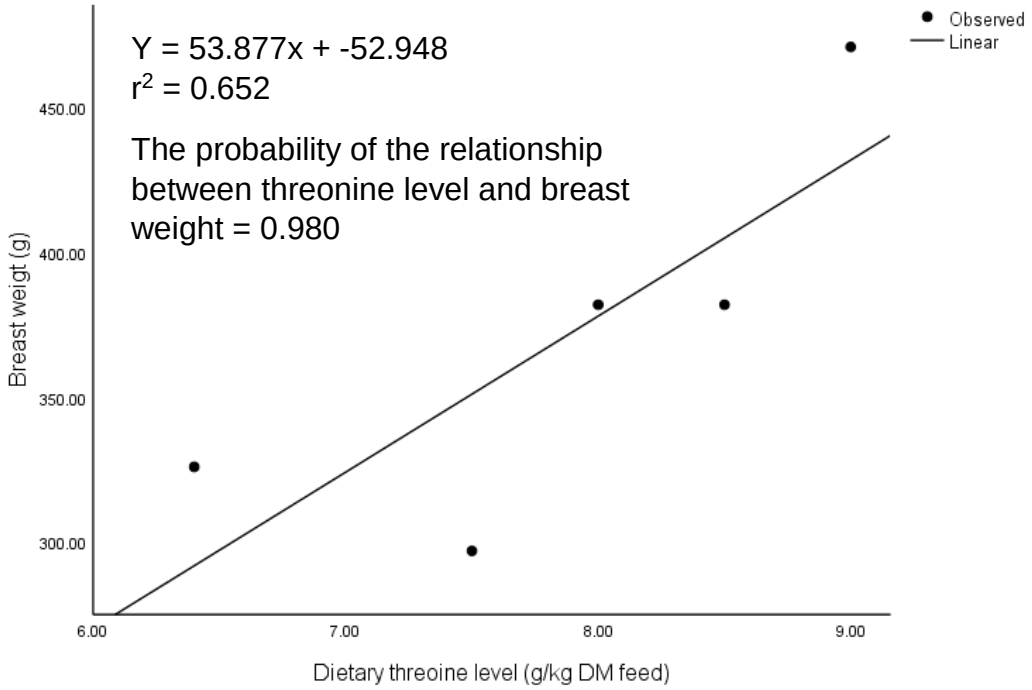

Figure 4.13 Relationship between dietary threonine level and breast weight of male Ross 308 broiler chickens aged 42 days

Results of the effects of dietary threonine level on breast meat tenderness, juiciness, flavour and shear force values of male Ross 308 broiler chickens aged 42 days are presented in Table 4.9. Dietary threonine level had no effect (P>0.05) on meat tenderness, juiciness and flavour of male Ross 308 broiler chickens aged 42 days. Meat shear force values of broiler chickens aged 42 days were not affected (P>0.05) by dietary threonine level.

Table 4.9 Effect of dietary threonine level on breast meat tenderness, juiciness, flavour and shear force values (kg) of male Ross 308 broiler chickens aged 42 days*

	Treatment #				
Variable	$MT_{6.4}$	$MT_{7.5}$	$MT_{8.0}$	$MT_{8.5}$	$MT_{9.0}$
Tenderness	3^a±0.3	3^a±0.2	3^a±0.3	4^a±0.3	3^a±0.1
Juiciness	3^a±0.1	3^a±0.2	3^a±0.0	3^a±1.9	3^a±0.3
Flavour	3^a±0.0	3^a±0.3	3^a±0.2	3^a±0.2	3^a±0.3
Shear force	14^a±1.8	15^a±1.0	15^a±0.4	14^a±0.5	15^a±0.2

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

a : Means with similar superscripts in the same row indicate non-significant differences between treatments (P>0.05).

: Treatment codes are explained in Chapter 3, Table 3.03 (The treatments were dietary threonine levels of 6.4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed).

CHAPTER 5

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Broiler chickens aged one to 21 days

The diets used in this study were isocaloric and isonitrogenous. All the diets contained similar nutrients except for threonine levels which ranged from 6.4 to 9.0 g/kg DM. Thus, any difference in response should be due to dietary threonine level. All the diets met the nutrient requirements for broiler chickens as specified by McDonald *et al*. (2011).

Increasing dietary threonine level from 6.4 to 9.0 g per kg DM did not improve feed intake of unsexed broiler chickens aged one to 14, and those aged 15 to 21 days. However, increasing dietary threonine level from 6.4 to 9.0 g per kg DM tended to decrease intake of unsexed broiler chickens aged 8 to 14 days. NRC (1994) recommended a threonine level of 8.0 g/kg DM for broiler chickens aged 1 to 21 days. Thomas (2008), recommended a threonine level of 6.9 g/kg DM for optimal intake of broiler chickens aged 14 to 28 days. Similarly, Samadi and Liebert (2006) reported a dietary threonine level of 7.9 g per kg DM for optimal intake of broiler chickens aged 1 to 21 days. It is concluded that the 6.4 g of threonine per kg DM observed in the present study as a level for optimal intake by the chickens is lower than those of 8.0, 6.9, 7.9 g/kg DM observed by NRC (1994), Thomas (2008) and Samadi and Liebert (2006), respectively.

Dietary threonine levels of 6.4 to 9.0 g per kg DM used in the present study had no effect on diet DM, CP, Fat, NDF, ADF and ash digestibilities in unsexed broiler chickens aged one to 21 days. However, Zhang *et al*. (2006) reported a dietary threonine level of 9.0 g/kg DM for optimal production in chickens aged one to 21 days. NRC (1994), recommended a dietary threonine level of 8.0 g/kg DM for optimal productivity in broiler chickens aged 1 to 21 days. Chen *et al*. (2017) observed a dietary threonine level of 8.8 g per kg DM for optimal production of broiler chickens aged 1 to 21 days.

The present study indicates that dietary threonine levels used affected ME intake and nitrogen retention values of unsexed Ross 308 broiler chickens aged one to 21 days. Dietary threonine levels of 8.0 and 9.0 g/kg DM resulted in chickens having higher metabolisable energy intake and nitrogen retention values, respectively. McDonald *et*

al. (2011) recommended a dietary threonine level of 8.3 g/kg DM for optimal intake in broiler chickens aged 1 to 21 days. De Filho *et al*. (2015) observed a dietary threonine level of 8.6 g/kg DM for optimal intake in broiler chickens aged 1 to 10 days.

Dietary threonine levels used in the present study did not improve growth rate of unsexed Ross 308 broiler chickens aged 1 to 7 days, and those aged 15 to 21 days. However, dietary threonine levels improved growth rate of unsexed Ross 308 broiler chickens aged 8 to 14 days. A dietary threonine level of 6.4 g/kg DM resulted in higher growth rates of unsexed Ross 308 broiler chickens aged 8 to 14 days. Increasing dietary threonine level from 7.5 to 9.0 g/kg DM decreased growth rates of unsexed Ross 308 broiler chickens aged 8 to 14 days. Ciftci and Ceylan (2004) reported that ideal total dietary threonine levels for growth performance were 6.8 to 7.5 g/kg DM for broiler chickens aged 1 to 21 days. Najafi *et al*. (2017) recommended a dietary threonine level of 9.7 g/kg DM for optimal growth rates of broiler chickens aged 1 to 21 days. Thus, a dietary threonine level of 6.4 g/kg DM observed in the present study for optimal growth rate is less than those of 6.8 to 7.5 g/kg DM and 9.7 g/kg DM observed by Ciftci and Ceylan (2004) and Najafi *et al*. (2017), respectively.

Dietary threonine levels used in the present study did not have any effect on feed conversion ratio of unsexed Ross 308 broiler chickens aged 1 to 7 or 15 to 21 days. However, dietary threonine levels affected feed conversion ratio of unsexed Ross 308 broiler chickens aged 8 to 14 days. A dietary threonine level of 9.6 g/kg DM optimized feed conversion ratio for unsexed Ross 308 broiler chickens aged 8 to 14 days. Kidd and Kerr (1997) reported a dietary threonine of 6.7 g/kg DM for better feed conversion ratios in broiler chickens aged 1 to 21 days. NRC (1994) observed a dietary threonine level of 7.4 g/kg DM for better feed conversion ratios in broiler chickens aged 1 to 21 days. Similarly, Valizade *et al*. (2016) and Kheiri and Alibeyghi (2017) observed dietary threonine levels of 8.4 and 9.0 g/kg DM, respectively, for better FCR values in broiler chickens aged 1 to 21 days.

Dietary threonine levels used in the present study affected live weights of unsexed Ross 308 broiler chickens aged 7, 14 or 21 days. Chickens on a diet having 6.4 g of threonine per kg DM tended to have higher live weights than those on the other treatments. Ng'ambi *et al*. (2017) observed a dietary threonine level of 6.3 g/kg DM for optimal live weights of slow-growing indigenous Venda chickens. Kidd *et al*. (2001)

recommended a dietary threonine level of 8.0 g/kg DM for optimal growth of broiler chickens aged one to 21 days. NRC (1994) recommended 8.0 g of threonine per kg DM diet for optimal live weights of chickens aged 1 to 21 days. Thus, the 6.4 g of threonine per kg DM of feed observed in the present study is lower than 8.0 g/kg DM of the diet observed by Kidd *et al.* (2001) and NRC (1994).

Dietary threonine levels used in the present study had no effect on gut organ digesta pH values of crop and small and large intestines of unsexed Ross 308 broiler chickens aged 21 days. However, dietary threonine levels used in the present study affected proventriculus, gizzard and caecum digesta pH values of chickens. The responses were variable; a dietary threonine level of 8.5 g/kg DM resulted in broiler chickens having higher gizzard digesta pH values. Dietary threonine level of 8.5 g/kg DM optimized proventriculus digesta pH values. Ng'ambi *et al*. (2017) observed that a dietary threonine level of 5.7 g/kg DM optimized digesta proventriculus pH values of female Venda chickens aged 91 days. The present finding of 8.5 g threonine per kg DM for optimizing gizzard digesta pH values is higher than that of 5.7 g/kg DM by Ng'ambi *et al*. (2017) for Venda chickens.

Dietary threonine levels used in the present study had no effect on small intestine lengths of unsexed Ross 308 broiler chickens aged 21 days. However, dietary threonine levels affected gut intestinal tract, caecum and large intestine lengths of unsexed Ross 308 broiler chickens. Dietary threonine levels of 6.6 and 8.4 g/kg DM optimized GIT and caecum intestine lengths of the chickens, respectively. Moghaddam *et al*. (2011) observed a dietary threonine level of 8.7 g/kg DM for optimal productivity of broiler chickens aged one to 14 days. Similarly, NRC (1994) recommended a dietary threonine level of 8.0 g/kg DM for optimal productivity for broiler chickens aged one to 21 days.

Dietary threonine levels used in the present study had no effect on small intestine weights of unsexed Ross 308 broiler chickens aged 21 days. However, dietary threonine levels affected crop, proventriculus, gizzard, caecum and large intestine weights of unsexed Ross 308 broiler chickens. The responses were variable. Increasing dietary threonine level from 6.4 to 9.0 g/kg DM tended to decrease crop and proventriculus weights of the chickens. However, increasing dietary threonine level from 6.4 to 9.0 g/kg DM increased caecum and large intestine weights of the

chickens. Kidd *et al*. (2001) recommended a dietary threonine level of 8.0 g/kg DM for optimal productivity of broiler chickens aged one to 21 days. Zaefarian *et al*. (2008) observed a dietary threonine level of 9.0 g/kg for optimal productivity of broiler chickens aged one to 21 days.

5.1.2 Broiler chickens aged 22 to 42 days

Dietary threonine levels of 6.4 to 9.0 g/kg DM used in this study did not have any effect on DM feed intake of male Ross 308 broiler chickens aged 22 to 28 days and those aged 36 to 42 days. However, dietary threonine levels affected DM feed intake of male Ross 308 broiler chickens aged 29 to 35 days, where diet DM intake tended to increase with increase in dietary threonine level. It is not clear why variable responses were observed in this study. NRC (1994) recommended a dietary threonine level of 7.4 g/kg DM for optimal intake in broiler chickens aged 29 to 35 days. However, Tugay *et al*. (2009) recommended a slightly higher threonine level of 7.5 g/kg DM for optimal DM intake in male broiler chickens aged 22 to 42 days. These values are within the threonine levels used in the present study.

Results of the present study indicate that dietary threonine levels used affected digestibility of the nutrients. A dietary threonine level of 8.0 g/kg DM improved diet DM, NDF, ADF and ash digestibilities in male Ross 308 broiler chickens aged 22 to 42 days. Similarly, Kidd and Kerr (1997) recommended a lower dietary threonine level of 7.5 g/kg DM for optimal diet DM digestibility in male broiler chickens aged 22 to 42 days. It is possible that the difference may be due to differences in threonine requirements by the broiler chicken breeds used in the experiments (NRC, 1994).

Dietary threonine levels used in the present study did not have any effect on nitrogen retention in male Ross 308 broiler chickens aged 22 to 42 days. However, dietary threonine levels used in the study affected metabolisable energy intake of male broiler chickens aged 22 to 42 days. A dietary threonine level of 8.0 g/kg DM improved metabolisable energy intake of male Ross 308 broiler chickens aged 22 to 42 days. Ng'ambi *et al*. (2017) observed a dietary threonine level of 6.2 g/kg DM for optimal nitrogen retention in slow growing female Venda chickens aged 8 to 13 weeks. McDonald *et al*. (2011) recommended dietary threonine levels of 7.3 and 6.5 g/kg DM

for optimal metabolisable energy intake and nitrogen retention in broiler chickens aged 4 to 6 weeks.

Dietary threonine levels of 6.4 to 9.0 g/kg DM used in the present study had no effect on growth rate of male Ross 308 broiler chickens aged 22 to 42 days. Kidd *et al*. (2004) and Kidd and Kerr (1997) recommended dietary threonine levels of 6.7 and 7.5 g/kg DM, respectively, for optimal growth rates of broiler chickens aged 22 to 42 days. Webel *et al*. (1996) recommended a lower dietary threonine level of 6.0 g/kg DM for optimal growth rate of broiler chickens aged 22 to 49 days. It is possible that dietary threonine levels used in the present study were above the requirements for growth of the chicken lines used, thus no response in growth was observed.

Dietary threonine levels used in the present study did not have any effect on feed conversion ratio of male Ross 308 broiler chickens aged 22 to 42 days. Kidd and Kerr (1997) recommended a threonine level of 7.5 g/kg DM for optimal feed conversion ratio in broiler chickens aged 22 to 42 days. Ng'ambi *et al*. (2017) observed a lower threonine level of 6.4 g/kg DM for optimal feed conversion ratio of slow-growing female Venda chickens aged 8 to 13 weeks.

Live weights of male Ross 308 broiler chickens aged 35 and 42 days were not affected by dietary threonine levels of 6.4 to 9.0 g/kg DM used in the present study. However, dietary threonine levels affected live weights of male Ross 308 broiler chickens aged 28 days. A dietary threonine level of 6.4 g/kg DM improved live weight of male Ross 308 broiler chickens aged 28 days. Increasing dietary threonine level from 7.5 to 9.0 g/kg DM did not improve live weights of the chickens. The results of the present study are similar to the findings of Kidd *et al*. (2004) who recommended a dietary threonine level of 6.5 g/kg DM for optimal live weights of broiler chickens aged 22 to 42 days.

Dietary threonine levels used in the present study did not affect crop, proventriculus, caecum, small and large intestine digesta pH values of male Ross 308 broiler chickens aged 42 days. However, dietary threonine levels of 6.4 to 9.0 g/kg DM affected gizzard digesta pH values of the chickens. Broiler chickens on a diet containing 9.0 g of threonine per kg DM had higher gizzard digesta pH values than those on a diet having 6.4 g of threonine per kg DM. Ng'ambi *et al*. (2013) observed a dietary threonine level of 5.7 g/kg DM for optimal gizzard digesta pH values in Venda chickens aged 91 days.

Dietary threonine levels used in the present study did not have any effect on small intestine, caecum and large intestine lengths of male Ross 308 broiler chickens aged 22 to 42 days. However, dietary threonine levels of 6.4 to 9.0 g/kg DM affected gut organ lengths of chickens. A dietary threonine level of 7.5 g/kg DM improved gut organ lengths of male broiler chickens. Lemme (2001) reported a dietary threonine level of 7.4 g/kg DM for optimal gut organ lengths of broiler chickens aged 21 to 42 days.

Gut organ weights of male Ross 308 broiler chickens aged 42 days were affected by dietary threonine levels of 6.4 to 9.0 g/kg DM. Broiler chickens on dietary threonine levels of 8.5 and 9.0 g/kg DM had higher crop weights than those on dietary threonine levels of 6.4, 7.5 and 8.0 g per kg DM. Increasing dietary threonine level from 6.4 to 9.0 g/kg DM increased small intestine weights of broiler chickens. Dietary threonine levels of 7.5 and 9.1 g/kg DM optimized proventriculus and large intestine weights of male Ross 308 broiler chickens aged 42 days. El-Faham *et al*. (2017) observed a threonine level of 8.7 g/kg DM for optimal gut organ weights of broiler chickens aged 21 to 42 days.

Dietary threonine levels used in the present study improved carcass organ weights of male Ross 308 broiler chickens aged 42 days. Male Ross 308 broiler chickens on a diet containing 9.0 g of threonine per kg DM had heavier carcass weights than those on diets containing 6.4 to 8.5 g of threonine per kg DM. Kheiri and Alibeyghi (2017), also, recommended a dietary threonine level of 9.0 g/kg DM for optimal carcass weights of broiler chickens aged 1 to 42 days. Khan *et al*. (2006) reported that supplementing diets with different levels of threonine did not have effects on carcass weights. Increasing dietary threonine level from 6.4 to 9.0 g/kg DM increased breast meat weights of male Ross 308 broiler chickens. The results of the present study are similar to the findings of Ciftci and Ceylan (2004) who reported that an increase in dietary threonine level increased breast meat yield of broiler chickens aged 22 to 42 days. Kidd *et al.* (2004) reported a threonine level of 8.7 g/kg DM diet for optimal breast meat weights of broiler chickens aged 21 to 42 days. Corzo *et al.* (2007) recommended a lower dietary threonine level of 7.9 g/kg DM for optimal breast meat weights of broiler chickens aged 15 to 42 days.

Dietary threonine level improved drumstick meat weights of male Ross 308 broiler chickens aged 42 days. Chickens on a dietary threonine level of 9.0 g/kg DM had

heavier drumstick meat weights than those on threonine levels of 6.4 to 8.5 g/kg DM. El-Faham *et al*. (2017) observed that a threonine level of 8.7 g/kg DM improved drumstick meat weights of broiler chickens aged 21 to 42 days. Abbasi *et al*. (2014) and Rezaeipour *et al*. (2012) recommended lower dietary threonine levels of 7.7 and 7.9 g/kg DM for optimal drumstick meat weights of broiler chickens aged 1 to 42 days, respectively.

Dietary threonine levels used in the present study improved thigh meat weights of male Ross 308 broiler chickens aged 42 days. Chicken on a dietary threonine level of 8.0 g/kg DM had heavier thigh meat weights than those on diets containing 7.5 or 8.5 g/kg DM. Corzo *et al*. (2007) and Kidd *et al*. (2004) recommended dietary threonine levels of 8.6 and 8.7 g/kg DM, respectively, for optimal thigh meat weights of broiler chickens aged 21 to 42 days.

Dietary threonine levels of 6.4 to 9.0 g/kg DM used in the present study had no effect on meat sensory attributes and shear force values of male Ross 308 broiler chickens aged 42 days. Lilly *et al*. (2011) observed no differences on sensory attributes and shear force values of broiler chickens fed different amino acid levels. Similarly, Ng'ambi *et al*. (2017) observed no effect of dietary threonine levels, ranging from 4 to 8 g/kg DM, on meat sensory attributes of female Venda chickens aged 50 to 91 days.

5.2 Conclusions and recommendations

Broiler chickens aged one to 21 days

Dietary threonine levels of 6.4 to 9.0 g/kg DM used in the present study affected feed intake, growth rate, live weights, metabolisable energy (ME) intake, nitrogen retention, feed conversion ratio and gut organ weights and lengths of unsexed Ross 308 broiler chickens aged one to 21 days. However, these variables were optimized at different threonine levels. This means dietary threonine level requirements will depend on the particular production variable. For example, a dietary threonine level of 9.6 g/kg DM optimized feed conversion ratio of unsexed Ross 308 broiler chickens aged 8 to 14 days, while a dietary threonine level of 6.6 g/kg optimized GIT lengths of unsexed broiler chickens. This has implications on ration formulation for broiler chickens. Thus, there is need to do more studies on the subject to ascertain the present findings.

Broiler chickens aged 22 to 42 days

Dietary threonine levels of 6.4 to 9.0 g/kg DM used in the present study affected feed intake, digestibility, metabolisable energy, live weight, digesta proventriculus pH values, GIT length, gut organ and carcass weights of male Ross 308 broiler chickens aged 22 to 42 days. However, these variables were optimized at different threonine levels. This means dietary threonine level requirements for Ross 308 broiler chickens will depend on the particular production variable of interest. Additionally, the threonine levels that optimized production parameters in the present study seem to be slightly higher than those in the literature. It is possible that dietary threonine requirement levels of the broiler chickens line used in the present study may be higher than for those used in the other experiments. Broiler chicken lines are constantly being improved through efficient breeding.

CHAPTER 6

REFERENCES

Abbasi, M., Mahdavi, A., Samie, A. and Jahanian, R. 2014. Effects of different levels of dietary crude protein and threonine on performance, humoral immune responses and intestinal morphology of broiler chicks*. Brazillian Journal of Poultry Science* 16(1): 35-44.

ADAS. 2016. Comparison of the Regulatory Framework and Key Practices in the Poultry Meat Supply Chain. UK Ltd in conjunction with the University of Arkansas.

Al-Hayani, W. 2017. Effect of threonine supplementation on broiler chicken productivity traits. *International Journal of Poultry Science* 16: 160-168.

Alvarado, C.Z. and Sams, A.R. 2004. Early postmortem injection and tumble marination effects on broiler breast meat tenderness. *Poultry Science* 83:1035-1038.

AOAC. 2010. Official Methods of Analysis, 19th edition. AOAC, International, North Frederic Avenue, USA.

AVEC. 2016. Annual Report 2016. Brussels, Belgium.

Balch, P.A. 2000. Prescription of Nutritional Healing, 3rd edition, United States of America: Avery Publishers.

Ball, R.O., Law, G., Bertolo, R.F.P. and Pencharz, P.B. 1999. Adequate oral threonine is critical for mucin production and mucosal growth by neonatal piglet gut. In: Proceedings of the VIIIth I nternational Symposium on protein Metabolism and Nutrition (G.E. Lobley, A. White, J.C. Macrae. eds), pp 31, EAAP Publication (vol 96).

Baker, D. 1985. Utilization of isomers and analogs of amino acids and other sulfur-containing compounds. Prog. *Food Nutritional Science* 10: 133-178.

Barkley, G. and Wallis, I. 2001. Threonine requirements of broiler chickens. *Journal of Poultry Science* 42: 610-615.

Belova, A.V., Smutka, L. and Rosochatecká, E. 2012. World chicken meat market - its development and current status. Acta University Agriculture Silvic Mendel Brun, Lx (2), 15-20.

Betts, M.J. and Russell, R.B. 2003. Amino Acid Properties and Consequences of Subsitutions. In: Gray, I.C., Ed., Bioinformatics for Geneticists Barnes MR, Wiley. http://dx.doi.org/10.1002/0470867302.ch14.

Bhargava, K.K., Hanson, R.P. and Sunde, M.L., 1971. Effects of threonine on growth and antibody production in chicks infected with Newcastle disease virus. *Journal of Poultry Science* 50: 710-713.

Boer, I.J.M., Van der Togt, P.L., Grossman, M. and Kwakkel, R. P. 2001. Nutrient flows for poultry production. *Journal of Poultry Science* 79: 172-179.

Bruns, K.W., Pritchard, R.H., and Boggs, D.L. 2004. The relationship among body weight, body composition, and intramuscular fat content in steers, *Journal of Animal Science* 82: 1315-1322.

Chee, S.H., Choct, M., Mikkelsen, L.L. and Kocher, A. 2010. Functional interactions of manno-oligosaccharides with dietary threonine in chicken gastrointestinal tract. II. Mucosal development, mucin dynamics and nutrient utilisation. *British Poultry Science* 51: 667-676.

Cheema, M., Qureshi, M.A. and Havenstein. G.B. 2003. A comparison of the immune response of a 2001 commercial broiler with a 1957 random bred broiler strain when fed representative 1957 and 2001 broiler diets. *Poultry Science* 82: 1519-1529.

Chen, Y., Cheng, Y., Li, X., Yang, W., Wen, C., Zhuang, S. and Zhou, Y.M. 2017. Effects of threonine supplementation on the growth performance, immunity, oxidative status, intestinal integrity, and barrier function of broilers at the early age. *Poultry Science* 96: 405-413.

Choct, M. 2009. Managing gut health through nutrition. *British Poultry Science* 50: 9-15.

Chrystall, B. 1994. Meat texture measurement. In: Quality Attributes and Their Measurement in Meat, Poultry and Fish Products. Pearson, A.M. and Dutson, T.R., (editions). Black Academic and Professional, UK, p. 316-336.

Ciftci, I. and Ceylan, N. 2004. Effects of dietary threonine and crude protein on growth performance, carcase and meat composition of broiler chickens. *British Poultry Science* 45: 280-289.

Corzo, A.M., Kidd, M.T., Dozier, W.A., Pharr, G.T. and Koutsos, E.A. 2007. Dietary threonine needs for growth and immunity of broilers raised under different litter conditions. *Poultry Science* 16: 574-582.

Dawson, D.L., Sheldon, B.W. and Milles, J.J. 1991. Effect of aseptic processing on the texture of chicken meat. *Poultry Science* 70: 2359-2367.

De Filho, A.L.B.M., de Oliveira, C.J.B., de Oliveira, H.B., Campos, D.B., Guerra, R.R., Costa, F.G.P. and Givisiez P.E.N. 2015. High incubation temperature and threonine dietary level improve ileum response against post-hatch *Salmonella Enteritidis* inoculation in broiler chicks. *Poultry Science* 10: 574-582.

Department of Agriculture, Forestry and Fisheries (DAFF), 2013. A profile of the South African broiler market value chain. Available at: http://www.daff.gov.za/daffweb3/Resource-Centre:1-51.

Dibner, J.J. 2003. Review of the metabolism of 2-hydroxy-4-(methylthio) butanoic acid. World Poultry Science Journal 59: 99-110.

DolezaL, G., Smith, H.C., Savell, G.W. and Carpenter, J.Z. 2006. Comparison of subcutaneous fat thickness, marbling and quality grade for predicting palatability of beef. *Journal of Food Science* 47: 397-401.

Dozier, I.I.I. W., Moran Jr, E. and Kidd M. 2001. Comparisons of male and female broiler responses to dietary threonine from 42 to 56 days of age. *Journal of Applied Poultry Research* 10: 53-59.

Dransfield, E. 1994. Tenderness of meat, poultry and fish. In: Quality Attributes and their Measurement in Meat, Poultry and Fish Products. Pearson, A.M. and Dutson, T.R., (editors). Black Academic and Professional, UK, pp 289-315.

El-Fahama, A. 2017. Effects of dietary threonine level, methionine sources, sex of broiler chicks and their interactions on carcass tissue distribution and chemical composition. *Egyptian Poultry Science Journal* 37: 155-167

Estalkhzir, F.M., Khojasteh, S. and Jafari, M. 2013. The effect of different levels of threonine on performance and carcass characteristics of broiler chickens. *Journal of Applied Science* 2: 382-386.

FAO. 2004. FAO Statistics. Food and Agriculture Organization, Rome, Italy; 2004. Available: http:www.apps.fao.org

FAO, IFAD and WFP. 2012. The State of Food Insecurity in the World 2012. *Rome*.

Fletcher, D.L. 2002. Quality of poultry meat, texture and colour. Proceedings of Georgia International Poultry Science Course, Athens, GA, pp 40-45.

Faure, M., Mettraux, C. Moennoz, D. Godin, J. Vuichoud, J. Rochat, F. Breuillé, D. Obled, C. and Corthésy-Theulaz, I. 2006. Specific amino acids increase mucin synthesis and microbiota in dextran sulfate sodium-treated rats. *Journal of Nutrition* 136: 1558-1564.

Gaddis, A. Hankins, O. and Hiner, R. 1950. Relationships between the amount and composition of press fluid, palatability and other factors of meat. *Food Technology* 4: 498-503.

Havenstein, G.B., Ferket, P.R. and Qureshi. M.A. 2003. Carcass composition and yield of 1957 vs 2001 broilers when fed representative 1957 and 2001 broiler diets. *Poultry Science* 82: 1509-1518.

Hoffmann, K. and Bucher, P. 1995. The FHA domain: A putative nuclear signalling domain found in protein kinases and transcription factors. *Trends in Biochemical sciences* 9: 347-349.

Howard, A. and Lawrie, R.A. 1956. Studies on beef quality. II. Physiological and biochemical effects of various pre-slaughter treatments. CSIRO Division of Food Preservation Technology Technical Paper No. 2. CSIRO Melbourne.

Hussein, A.S., Cantor, A.H., Pescatore, A.J., Gates, R.S., Burnham, D., Ford, M.J. and Paton, N.D. 2001. Effect of low protein diets with amino acid supplementation on broiler growth. *Jounal of Applied Poultry Research* 10: 354-362.

Juliano, B.O. 1993. Rice in human nutrition. FAO, Rome. pp.17-34.

Kheiri, F. and Alibeyghi, M. 2017. Effect of different levels of lysine and threonine on carcass characteristics, intestinal microflora and growth performance of broiler chicks. *Itallian Journal of Animal Science* 16: 580-587.

Kidd, M.T., Corzo, A., Hoehler, D., Kerr, B.J., Barber, S.J. and Branton, S.L. 2004. Threonine needs of broiler chickens with different growth rates. *Poultry Science* 83:1368-1375.

Kidd, M.T., Barber, S.J., Virden, W.S., Dozier, W.A., Chamblee, D.W. and Wiernusz, C. 2003. Threonine responses of Cobb male finishing broilers in differing environmental conditions. *Journal of Applied Poultry Research* 9: 223-233.

Kidd, M.T. 2000. Nutritional consideration concerning threonine in broilers. *World Poultry Science Journal* 56: 139-151.

Kidd, M.T., Lerner, S.P., Allard, J.P., Rao, S.K. and Halley, J.T. 1999. Threonine needs of finishing broilers: growth, carcass and economic responses. Journal of Applied Poultry Research 8: 160-169.

Kidd, M.T. and Kerr, B.J. 1997. Threonine responses in commercial broilers at 30 to 42 days. *Poultry Science* 6: 362-367.

Kidd, M.T. and Kerr, B.J 1996. Threonine for poultry. *Jounal of Applied Poultry Research* 5: 358-367.

Lawrie, R. A. 1998. Lawrie's Meat Science. 6th edition. Woodhead Publishing Limited, Cambridge, England.

Legninger, A.L., Nelson, D.L. and Cox, M.M. 2000. Principles of Biochemistry. 2nd edition. Worth Publishers Incorporation, New York, pp 558-560.

Lemme, A. 2003. Reassessing amino acid levels for Pekin ducks. *International Journal of Poultry Science* 42: 18-24.

Mack, S., Bercovici, D., De Groote, G., Leclercq B., Lippens, M., Pack, M., Schutte, J.B. and Van Cauwenberghe, S. 1999. Ideal amino acid profile and dietary lysine specification for broiler chickens of 20 to 40 days of age. *British Poultry Science* 40: 257–265.

Maroufyan, E., Kasim, A., Hashemi S.R., Loh T.C., Bejo, M.H. and Davoodi. H. 2010. The effect of methionine and threonine supplementations on immune responses of broiler chickens challenged with infectious bursal disease. *American Journal of Applied Sciences* 7:44-50.

Mbajiorgu, C.A. 2010. Effect of dietary energy to protein ratio levels on growth and productivity of indigenous Venda chickens raised in closed confinement from one day up to 13 weeks of age. PhD Thesis, Animal Production Department, University of Limpopo

McCormick, R. J. 1994. Structure and properties of tissues. In D. M. Kinsman, A. W. Kotula, & B. C. Breidenstein (Eds.), Muscle Foods. Meat, Poultry and Seafood Technology pp. 25–62. New York: Chapman and Hall.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., Sinclair, L.A. and Wilkinson, R.G. 2011. Animal Nutrition. 7th edition, United Kingdom, Prentice Hall publishers, pp 656.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D. and Morgan C.A. 2010. Animal Nutrition. Pearsoned Education Limited, Sixth Edition, London.

McPeake, C. A. 2001. USDA marbling and carcass physiological maturity related differences for beef tenderness and palatability characteristics. PhD Thesis, Oklahoma State University.

Meilgaard, M.C., Civille, G.V. and Bernard, C. 2016. Sensory Evaluation Techniques. 10.1201/9781439832271.

Moghaddam, H.S., Moghaddam, H.N., Kermanshahi, H., Mosavi, A.H., Raji, A. 2011. The effect of threonine on mucin2 gene expression, intestinal histology and performance of broiler chicken. *Itallian Journal of Animal Science* 10: 66-71.

Moreki, J., Dikeme, C. and Poroga, B. 2010. The Role of Village Poultry in Food Security and HIV/AIDS Mitigation in Chobe District of Botswana. *Livestock Resources Rural Development* 22: 5-10.

NAFIS. 2017. Feed and Feedings. http://www.nafis.go.ke/livestock/poultry-chicken/general-information/feeds-and-feeding/.

Najafi, R., Ahmar, R., Tazehkand, G. 2017. Effect of different dietary threonine levels on optimal growth performance and intestinal morphology in 1-14 days old Ross 308 broilers. *Brazillian Journal of Poultry Science* 19(3): 59-66.

Ndirangu, J., Newell, M.L., Tanser, F., Herbst, A.J. and Bland, R. 2010. Decline in early life mortality in a high HIV prevalence rural area of South Africa, p. 593-602 (vol. 24).

Ng'ambi, J. Ramuthaga, N. and Brown, D. 2017. Effect of dietary threonine level on productivity and carcass characteristics of indigenous Venda chickens. *Indian Journal of Animal Research* 10: 32-44.

NRC. 1994. Nutrient Requirements of Poultry. 9th Revised Edition. National Academy Press: Washington, DC.

Ojano-Dirain, C.P. and Waldroup, P.W. 2002. Evaluation of lysine, methionine and threonine needs of broilers from three to six weeks of age under moderate temperature stress. *International Journal of Poultry Science* 1: 16-21

Pavelková, A., Kačániová, M., Hleba, L., Petrová, J., Pochop, J. and Čuboň, J. 2013. Sensory evaluation of chicken breast treated with oregano essential oil. Scientific Papers: *Animal Science and Biotechnologies* 46: 379-383.

Pandurevic, T., Mitrovic, S., Ristanovic, B. and Stanisic, V. 2014. Quality of chicken meat from conventional and organic production. Proceedings of the 5th International Scientific Agricultural Symposium East Sarajevo, Jahorina, Faculty of Agriculture, pp. 849-853.

Rama Rao, S.V., Praharaj, N.K., Reddy, M.R and Sridevi, B. 1999. Immune competence, resistance to Escherichia coli and growth in male broiler parent chicks fed different levels of crude protein*. The Vetenary Research Committee* 23: 323-336.

Rao, S.R., Raju M., Panda A., Poonam N., Moorthy O., Srilatha T., Sunder S. (2011). Performance, carcass variables and immune responses in commercial broiler chicks fed graded concentrations of threonine in diet containing sub-optimal levels of protein. *Animal Feed Science and Technology* 169(3): 218-223.

Rehman, H.R., Ghumro, P.B., Dino, G., Khan, S.H., Hussain, Z., Ahmed, S. and Hameed, A. 2012. Substitution of crystalline l-lysine with l-lysine enriched fermentation broth in feed and effect on the performance of broiler chicks. *Journal of Applied Animal Research* 40: 118-123.

Renand, G., Larzul, C., Le Bihan-Duval, E. and Le Roy, P. 2003 Genetic improvement of meat quality in the different livestock species: Present situation and prospects. *Animal Production* 16: 159-73.

Rezaeipour, V. and Gazani S. 2014. Effects of feed form and feed particle size with dietary L-threonine supplementation on performance, carcass characteristics and blood biochemical parameters of broiler chickens. *Journal of Animal Science* 56: 20-24.

Richard, J.J. 2005. Production and growth-related disorders and other metabolic diseases of poultry: A review. *The Veterinary Journal* 169: 350-360.

RIRDC. 2014. Rural Industries Research and Development Corporation Chicken. meat program five-year RD&E plan 2014-2019. Available at: http://www.rirdc.gov.au/research-programs/animal-industries/chicken-meat.

Ristic M., Miscevic B. (2012): Survey of the tenderness of meat of beef, pork poultry and rabbit – a comparison. Fleischwirtschaft Frankfurt 92: 100-104.

Rostagno, H.S, Pupa, J.M. and Pack M. 1995. Diet formulation for broilers based on total versus digestible amino acids. *Journal of Applied Poultry Research* 4: 293-299.

Samadi, D. and Liebert, F. 2006. Modeling of threonine requirement in fast-growing chickens, depending on age, sex, protein deposition, and dietary threonine efficiency. *Poultry Science* 85: 1961-1968.

SAPA. 2012. South African Poultry Association. Broiler organisation committee chairperson report 2011.

SAPA. 2013. Provincial Distribution of Chickens in South Africa. South African Poultry Association, SA.

SAPA. 2016. Broiler Industry Stats Summary. South African Poultry Association.

SAS. 2008. Statistical Analysis System. User's Guide: Statistics, 11th Edition. SAS Institute, Inc. Raleigh, North Caroline, USA.

Savell, J.W., and Cross, H.R. 1988. The role of fat in the palatability of beef, pork, and lamb. In: Designing Foods: Animal Product Options in the Marketplace, pp 345-355, National Academy Press, Washington, DC.

Savell, J.W., Cross, H.R., Francis, J.J., Wise, J.W., Hale, D.S. and Wilkes, D.L. 1989. National Consumer Retail Beef Study: Interaction of trim level, price and grade on consumer acceptance of beef steaks and roasts. *Journal of Food Quality* 12: 251-274.

Schutte, J.B. and Pack, M. 1995. Sulfur amino acid requirements of broiler chicks from fourteen to thirty-eight days of age. 1. Performance and carcass yield. *Poultry Science* 74: 480-487.

Sikes, A., Tornberg, E. and Tume, R. 2010. A proposed mechanism of tenderising post-rigor beef using high pressure-heat treatment. *Meat Science* 84: 390-399.

Shiringani, R.P. 2007. Effect of planting date and location on phenology, yield and yield components among cowpea selected cowpea varieties. Masters dissertation, University of Limpopo, South Africa. 20-73.

Smith, L.C. and Wiseman, D. 2007. Is food security more severe in South Asia or sub-Saharan Africa? International Food Policy Research Institute Discussion Paper, 712, 52. Available at: http://ageconsearch.umn.edu/bitstream/42401/2/ifpridp00712.

Smith, D.P. and Acton, J.C. 2001. Marination, cooking and curing of poultry products. In: Poultry Meat Processing. Edited by A.R., Sams. CRC Press, Boca Raton, FL. pp 257-280.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M. and De Haan, C. 2006. Livestock's Long Shadow, FAO, *Rome*.

Strakova, E., Večerek, V., Suchy, P. and Vitula, F. 2003. The Comparison of Carcass Quality in Fattening Chicks and Pheasants, pp 83-87.

Takahashi, K. 1996. Structural weakening of skeletal muscle tissue during post-mortem ageing of meat: the non-enzymatic mechanism of meat tenderization. *Meat Science* 43: 67-80.

Tallentire, C.W., Leinonen, I. and Kyriazakis, I. 2016. Breeding for efficiency in the broiler chicken: A 719 review. *Agronomy for Sustainable Development* 36: 66-69.

Temim, S., Chagneau, A.M., Guillaumin, S., Michel, J., Peresson, R., Geraert, P.A. and Tesseraud, S. 1999. Effects of chronic heat exposure and protein intake on growth performance, nitrogen retention and muscle development in broiler chickens. Reproduction. *Nutritional Development* 39: 145-156.

Thompson, J.M. 2004. The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. *Australian Journal of Experimental Agriculture* 44: 645-652.

Trevisi, P., Corrent, E., Mazzoni, M., Messori, S., Priori, D., Gherpelli, Y., Simongiovanni, A. and Bosi. P. 2015. Effect of added dietary threonine on growth performance, health, immunity and gastrointestinal function of weaning pigs with differing genetic susceptibility to Escherichia coli infection and challenged with E. coli K88ac. *Journal Animal Physiology of Animal Nutrition* 99: 511-520.

Valizade, M.R., Sadeghi, A.A., Chamani, M., Shawrang, P. and Kashan N. 2016. The effects of increase in threonine to lysine ratio on performance, blood parameters and humoral immune responses of male broiler chickens challenged with *Salmonella*. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi* 22(2): 165-172.

Varnam, A.H. and Sutherland, J.P. 1995. Meat and Meat Products: Technology, Chemistry, and Microbiology. Chapman and Hall, London.

Walsh, M., Devereaux, P.J. and Garg, A.X. 2013. Relationship between Intraoperative Mean Arterial Pressure and Clinical Outcomes after Noncardiac Surgery: Toward an Empirical Definition of Hypotension. *Anesthesiology* 119: 507-515.

Wang, S.Z., Hu, X.X., Wang, Z.P., Li, X.C. and Tang, Z.Q. 2012. Quantitative trait loci associated with body weight and abdominal fat traits on chicken chromosomes 3, 5 and 7. *Genetic Molecular Research* 11: 956-965.

Warris, P. D. 2000. Meat Science: An Introductory Text. New York: CABI Pub. Inc.

Webel, D.M., Fernandez, S.R., Parsons C.M. and Baker D.H. 1996. Digestible threonine requirement of broiler chickens during the period three to six and six to eight weeks post-hatching. *Poultry Science* 75: 1253-1257.

Wood, J.D. 1990. Consequences for meat quality of reducing carcass fatness. In: Reducing Fat in Meat Animals. Elsevier Applied Science, Essex, England. pp. 344-397.

Wood, J.D., Nute, G.R., Fursey, G.A.J. and Cuthbertson, A. 1995. The effect of cooking conditions on the eating quality of pork. *Meat Science* 40: 127-135.

Wood, J.D. and Enser, M. 1997. Factors Influencing Fatty Acids in Meat and The Role of antioxidants in improving meat quality. *British Journal of Nutrition* 78 (1): 549-550

Wils-Plotz, L. and Dilger, E. 2013. Combined dietary effects of supplemental threonine and purified fiber on growth performance and intestinal health of young chicks. *Poultry Science* 92: 726-34.

Xiong, Y.L. 2005. Role of myofibrillar proteins in water-binding in brine-enhanced meats. *International Food Research* 38:281-287.

Zaefarian, F., Zaghari, M. and Shivazad, M. 2008. The threonine requirements and its effects on growth performance and gut morphology of broiler chicken fed different levels of protein. *International Journal of Poultry Science* 7(12): 1207-1215.

Zaghari, M., Zaefarian, F. and Shivazad, M. 2011. Standardized ileal digestible threonine requirements and its effects on performance and gut morphology of broiler chicks fed two levels of protein. *Journal of Agricultural Science and Technology* 13: 541- 552.

Zhang, A.W., Lee, B.D., Lee, S.K., Lee, K.W., An, G.H., Song, K.B. and Lee, C.H., 2005. Effects of yeast *(Saccharomyces cerevisiae)* cell components on growth performance, meat quality, and ileal mucosa development of broiler chicks. *Poultry Science* 84: 1015-1021.

Zhang, Q., Chen, X., Eicher, S.D., Ajuwon, K.M. and Applegate, T.J. 2016. Effect of threonine deficiency on intestinal integrity and immune response to feed withdrawal

combined with coccidial vaccine challenge in broiler chicks. *British Journal of Nutrition* 116(12): 2030-2043.