Evaluation of threonine inclusion in a diet on productivity and carcass characteristics of indigenous Boschveld chickens

by

Tebogo Mack Sekgotodi

A RESEARCH MINI-DISSERTATION

Submitted in fulfilment of the requirements for the degree of

Master of Science (ANIMAL PRODUCTION)

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

Supervisor: DR TG Manyelo

Co-supervisor: PROF JW Ng'ambi

December 2024

DECLARATION

I declare that this research report 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 work in design and execution, and that all materials contained herein have been duly acknowledged.

Signature:  01/12/2023

Tebogo Mack Sekgotodi

ACKNOWLEDGEMENTS

I would like to thank Almighty God for honoring me with the strength to persevere and endure despite the overwhelming obstacles throughout this study. First and foremost, I thank Dr TG Manyelo and Prof JW Ng'ambi, as my supervisor and co-supervisor for accepting me into their group. They inspired, guided, supported, and directed me to carry out this project. I would, also, like to thank the National Research Foundation (NRF) for financial support during the period of my study.

Additionally, I acknowledge my classmate TT Magonyane, Livestock Unit Manager and general workers, Animal Production Senior Laboratory Technician (Ms. NP Nhleko), Aquaculture general workers and Technician (Mr. G Geldenhuys) for assistance during this study, and my research team Bachelor of Science in Agriculture (Animal Production) final year students for their contribution during data collection.

I wish to extend my special appreciation to my family and extended family for their love, support, encouragement, and prayers during the entire period of my study.

DEDICATIONS

I dedicate this work to my late father (Matome Solomon Sekgotodi), my mother (Ramokone Annah Sekgotodi), my late daughter (Segopotso Amanda Mailula), my late grandparents and myself.

ABSTRACT

Protein ingredients are the most expensive inputs in poultry production even though not included in large quantities compared to energy ingredients. The study aimed to evaluate dietary threonine levels for optimal production and carcass characteristics of indigenous Boschveld chickens. Two experiments were conducted to determine the effect of dietary threonine levels on the productivity and carcass characteristics of indigenous Boschveld chickens. The first part of the study was to determine the effect of threonine inclusion level in a diet on feed intake, feed conversion ratio, body weight gain and gut organ measurements of unsexed indigenous Boschveld chickens aged 1 to 49 days. A total of 200-day-old chicks, weighing 30 ± 5g per bird, were randomly allocated to 5 dietary treatment levels in a completely randomized design (CRD), replicated four times with 10 chicks per replicate. The experimental diets were isocaloric and isonitrogenous, and were made to fulfil the necessary nutritional standards, but with different threonine inclusion levels of 4, 7.5, 8, 8.5, or 9 g per kg DM of diet. Data were analysed using Statistical Analysis System version 9.4 and it was subjected to one-way analysis of variance (ANOVA). A quadratic equation was used to determine dietary threonine levels for optimal duodenum pH and duodenum weights of unsexed indigenous Boschveld chickens aged 49 days. Threonine inclusion levels had an effect ($p<0.05$) on feed intake, body weight gain, and feed conversion ratio of unsexed indigenous Boschveld chickens aged 1-49 days. Threonine inclusion levels had no effect ($p>0.05$) on crop, gizzard, ileum, jejunum, caeca, large intestine pH values and caeca lengths of unsexed indigenous Boschveld chickens aged 49 days. However, threonine inclusion levels had effect ($p<0.05$) on duodenum pH, GIT, duodenum, large intestine lengths, crop, gizzard, ileum jejunum, and caeca weights of unsexed indigenous Boschveld chickens aged 49 days. Feed conversion ratio, duodenum pH values and duodenum weights of unsexed indigenous Boschveld chickens were optimized at dietary threonine level of 12.00, 0.70 and 8.00 g/kg DM, respectively.

The second part of the study was to determine the effect of threonine level in a diet on carcass characteristics and quality of male indigenous Boschveld chickens aged 50 to 91 days. A total of 75 male chickens weighing 600 ± 10g were used in a completely randomized design having 5 treatment groups, replicated three times, with five

chickens per replicate. The experimental diets were formulated to be isocaloric and isonitrogenous and meet the nutritional requirements, but with different threonine inclusion levels of 4, 7.5, 8, 8.5, or 9 g per kg DM of feed. Data were analysed using Statistical Analysis System version 9.4 and was subjected to one-way ANOVA. A quadratic equation was used to determine dietary threonine levels for optimal large intestine lengths, crop, and jejunum weights of male indigenous Boschveld chickens aged 91 days. Threonine inclusion levels had an effect ($p<0.05$) on feed intake, body weight gain (BWG), and feed conversion ratio (FCR) of male indigenous Boschveld chickens aged 50 to 91 days. The BWG, FCR, crop, jejunum, and large intestine lengths of male indigenous Boschveld chickens were optimized at dietary threonine levels of 6.12, 6.87, 5.95, 5.66, and 6.08 g/kg respectively. Threonine inclusion levels had no effect ($p>0.05$) on crop, gizzard, ileum, jejunum, duodenum, caeca, large intestine pH values, GIT, caeca lengths, large intestine weights, juiciness, flavour, overall acceptability, and cooking loss of indigenous Boschveld chickens aged 91 days. However, dietary threonine inclusion levels had an effect ($p<0.05$) on large intestine lengths, crop, and jejunum weights, carcass, thigh, drumstick, breast, and abdominal fats weights, and tenderness of male indigenous Boschveld chickens aged 91 days.

It is concluded that dietary threonine levels used in this study affected the production performance of indigenous Boschveld chickens aged 1-49 more than indigenous Boschveld chickens aged 50-91 days. However, production variables were optimized at different dietary threonine levels. This has implication on diet formulation for the chickens. Thus, there is a need for further studies on the subject to ascertain the present findings.

Keywords: Threonine, indigenous Boschveld chickens, feed intake, body weight gain, feed conversion ratio, carcass characteristics, gut organ.

TABLE OF CONTENTS

LIST OF TABLES

S

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	Acid Detergent Fibre
ANOVA	Analysis of Variance
AOAC	Association of Official Agricultural Chemists
ATM	Threonine inclusion level in male
ATU	Threonine inclusion level in unsexed
AVMA	American Veterinary Medical Association
BWG	Body weight gain
Ca	Calcium
Co	Cobalt
Cu	Copper
CRD	Completely Randomized Design
DM	Dry matter
ESCs	Embryonic stem cells
Fe	Iron
FCR	Feed conversion ratio
FI	Feed intake
DDM	Duodenum
DM	Dry matter
g/kg	Grams per kilograms

GIT	Gastrointestinal tract
IQF	Individually quick frozen
pH	Potential of hydrogen
LC-PUFA	Long-chain polyunsaturated fatty acid
LI	Large intestine
ME	Metabolisable energy
Mg	Magnesium
Mo	Molybdenum
Mn	Manganese
MUFA	Monosaturated fatty acids
NACC	National Agricultural Catchments Council
NDF	Neutral Detergent Fibre
NLIN	Nonlinearity
NRC	National Research Council
NRF	National Research Foundation
SAS	Statistical Analysis System
Se	Seleium
SE	Standard error
SFA	Saturated fatty acids
SSF	Solid-state fermentation
USDA	United States Department of Agriculture

W_0	Weight before cooking
W_1	Weight after cooking
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background

In broiler chickens' depression of the growth rate, feed intake, and carcass yield are consequences of threonine deficiency, whereas the effects in laying birds are a low laying rate, egg weight, egg mass, and feed conversion ratio (Azzam *et al*., 2011; Zhang *et al*., 2014; Fouad *et al*., 2017). Threonine is required to improve the secretion of digestive enzymes and to maintain normal populations of useful bacteria when the diet contains a low level of crude protein (Dong *et al*., 2017). Thus, threonine can maintain productivity when birds are fed diets with low crude protein contents. Threonine is a nutritionally important amino acid because poultry cannot synthesize it on their own (NRC, 1994). Threonine is a crucial part of body protein and functions as a precursor to lysine and serine (NRC, 1994). Previous studies have highlighted the critical effect of threonine supplementation on improving egg production in poultry. Furthermore, threonine supplementation improves the performance of chickens and enhances the morphological and functional development of the intestinal mucosa at hatching (Moreira Filho *et al*., 2019). 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 based on digestible amino acids (Maharjan *et al*., 2021).

1.2 Problem statement

As mentioned earlier, threonine is a crucial part of body protein and functions as a precursor to lysine and serine (NRC, 1994). These amino acids (threonine, lysine, and serine) are necessary for the development and softness of meat (Ng'ambi *et al*., 2017). Currently, data on the appropriate threonine requirements for genotypes of slow-growing chickens is limited and inconclusive. Thus, dietary threonine level requirements for slow-growing indigenous chicken breeds such as Boschveld chickens are not available. As a result, it is challenging to formulate diets with threonine levels that meet the needs of these slow-growing chickens for maximum output. The growth rate, carcass yield, and feed conversion ratio (FCR) of chickens fed a diet low in threonine are low (Kidd *et al*., 2004; Dozier *et al*., 2001; NRC, 2004). Amino acids are vital for both growth and meat tenderness (NRC, 1994). According to Kidd and Kerr (1996), the impact of dietary threonine on chicken productivity has been inconsistent, ranging from 5.8 to 7.9 g/kg DM.

1.3 Motivation of the study

This study will provide information on digestibility, growth rate, feed intake, and carcass characteristics responses of indigenous Boschveld chickens fed different dietary levels of threonine. The data will help in improving the growth rate, immune system responses, and meat quality of indigenous Boschveld chickens, and will be valuable to poultry farmers and other stakeholders that aim at enhancing the productivity of indigenous Boschveld chickens.

1.4 Aim of the study

The study aims at evaluating dietary threonine levels for optimal production and carcass characteristics of indigenous Boschveld chickens.

1.5 Objectives

The objectives of this study were to determine:

i. The effect of dietary threonine levels on feed intake, FCR, and body weight gain (BWG) of indigenous Boschveld chickens.
ii. The effect of dietary threonine levels on carcass quality (meat pH, meat colour, tenderness, juiciness, and flavour) of indigenous Boschveld chickens aged 50 to 91days.
iii. The effect of dietary threonine levels on gut organ characteristics (lengths, weights, and pH) of indigenous Boschveld chickens aged 50 to 91days.

1.6 Hypotheses

The following are the null hypotheses of the study:

i. Dietary threonine inclusion levels have no effect on feed intake, FCR, and BWG of indigenous Boschveld chickens.
ii. Dietary threonine inclusion levels have no effect on carcass quality (meat pH, meat color, tenderness, juiciness, and flavor) of indigenous Boschveld chickens aged 50 to 91 days.
iii. Dietary threonine inclusion levels have no effect on gut organ characteristics (lengths, weights, and pH) of indigenous Boschveld chickens aged 50 to 91days.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Smallholder farmers in Southern Africa and many parts of the developing world generally rely on chicken meat to meet their dietary protein requirements (Azimu *et al*., 2018). Most of these farmers breed and keep indigenous chickens, which are mostly tolerant of local diseases and parasites; moreover, they provide huge economic benefits and income for rural communities. The sudden increase in the demand for natural or organic meat could have influenced smallholder farmers to consider farming with indigenous chickens because these chickens need minimal use of additives and chemicals (Selaledi *et al*., 2021). The indigenous chickens are known to be economically, socially, and culturally important to the people of Africa and other developing countries, especially those from poor communities (Manyelo *et al*., 2020). Although they are associated with poor productivity, most consumers prefer their meat flavour (Manyelo *et al*., 2020). However, conventional protein sources, such as soybean and fishmeal, which are normally used in poultry diets, are expensive, thus, the need for the search for alternative sources of protein (Ramos-Elorduy, 2017).

Due to high feed costs, the formulation of diets that meet nutrient requirements for optimal productivity of indigenous chickens has lagged (Atela *et al.,* 2019). Perhaps, this might be because energy and protein sources are the second-largest components of poultry diets and the most expensive (Iji *et al*., 2017). This then defeats the purpose of keeping indigenous chickens in a low-resource setting. Threonine is the third limiting amino acid after methionine and lysine in the diets of broiler chickens, it is, thus, very important in poultry nutrition. The dietary amino acid concentration must meet the maintenance and tissue accretion needs of poultry, especially towards the middle and end of the growth period. Nutrition is the backbone of profitable broiler production and on average, it accounts for about 80–90% of the total cost of production (NAFIS, 2017). Modern broilers can potentially attain 2 kg of body weight by consuming 3 kg of feed within 5 weeks (Zampiga *et al.,* 2018).

2.2 Biochemical functions of threonine

Threonine, otherwise known as α-amino-β-hydroxybutyric acid, is an indispensable amino acid must be obtained from the diet. It is identified as the third limiting amino acid in broilers. Threonine is an important bioactive molecule that has vital mediation effects on protein synthesis, energy metabolism, and nutrient absorption (Chen *et al*.,

2017). It has been reported that appropriate dietary threonine levels can promote animal growth, enhance immune function, and maintain intestinal health (Chen *et al*., 2018). Threonine is an efficient nutritional modulator affecting nutrition metabolism. Threonine supplementation has been shown to enhance hepatic lipid metabolism, and its deficiency may induce hepatic triglyceride accumulation (Jiang *et al.,* 2017). Threonine exerts a protective effect on lipid metabolic disorders by regulating lipogenesis signalling pathway and the expression of thermogenic genes (Ma *et al*., 2020). More importantly, a limited number of studies have indicated that threonine plays an indispensable role in physiological regulation in many processes besides simple nutrition, particularly in cell growth and proliferation, including in embryonic stem cells (ESCs) (Kim *et al*., 2021).

2.3 The effect of threonine on chicken productivity

The growth rates of chickens depend on the availability of an adequate amount of amino acids that can be used to synthesise proteins involved in many physiological processes, including signal transduction, hormone signalling, cell structure, and antioxidant systems (Chen *et al.,* 2016). Amino acids are also important for energy metabolism, urinary system function, and sexual maturation (Geraert and Mercier, 2016). Due to the rapid growth of commercial chicken strains, the availability of amino acids for optimal growth, particularly muscle growth, as well as, physiological function is critical (Chen *et al*., 2016). In addition, the aliphatic amino acid threonine is an important growth factor in broilers and is one of the basic amino acids needed for the growth of domesticated birds (Qaisrani *et al.,* 2018). Chickens cannot synthesise several essential amino acids including threonine thus threonine supplementation is required (Elnesr *et al.,* 2019, 2020). Amino acids play many important physiological roles, particularly in thyroid function and a diet lacking threonine, as well as, other amino acids can lead to diminished physiological function (Bortoluzzi *et al.,* 2018; Debnath *et al.,* 2019). Moreover, threonine helps maintain the integrity of the intestinal mucosal barrier and thus can enhance nutrient uptake by broiler chickens (Corzo *et al*., 2017). The addition of threonine to broiler chicken diets increases productivity in terms of body weight, feed conversion, dressing percentage, relative breast weight, and thigh (Rezaeipour, 2015). Rezaeipour *et al.* (2015) reported that threonine supplementation together with feed particle size improved FCR, whereas threonine supplementation for the first 42 days after birth improved FCR to levels above those

seen with the addition of probiotics. Below is table 2.01 of dietary threonine requirements for optimum feed intake, weight gain, feed conversion ratio, and breast muscle weight of broiler chickens at different ages.

Table 2.01 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	FI	Weight gain	FCR	Breast	Authors
1-14 days	0.65	-	0.89	-	Najafi *et al.* (2017)
1-42 days	3.2	1.7	1.4	0.79	Kheiri and Alibeyghi (2017)
1-42 days	1.6	6.0	2.8	-	Min *et al.* (2017)
1-42 days	1.0	0.6	2.8	-	Chen *et al.* (2017)
1-42 days	-	1.0	4.7	-	Valizade *et al.* (2016)
21-42 days	0.72	0.73	0.77	0.74-0.71	NRC (1994)

2.4 Flavour, tenderness, and juiciness of chicken meat

2.4.1 Flavour

Flavour is the sensation that results primarily from the chemical senses of taste, smell, and the trigeminal system (Spence, 2013). Gustation, or taste, is perceived when a taste-active compound binds to specific proteins known as taste receptors (Gravina *et al*., 2013). This event leads to cell membrane depolarisation, neurotransmitter release, and the eventual propagation of sensory information to the central nervous system via an action potential (Boughter Jr. and Munger, 2013). Findings of animal nutrition researchers conducted over years have shown that several pre- and post-mortem factors affect the flavour of chicken meat. Breed/strain of the chicken, diet, presence of free amino acids and nucleotides, irradiation, high pressure treatment, cooking, antioxidants, pH, sex, and ageing are considered as the main determinants of flavour (Jayasena *et al.,* 2013). Although the number of identified volatile compounds in meat is very large, only few have been shown to contribute meaty aromas (Kosowska *et al.,* 2018). Meat flavour volatiles such as buttery, caramel, roast, burnt, sulphurous, green, fragrant, fatty, and nutty, and have thus been designated as aroma modifiers, and the majority are considered relatively not important. The most

important meat flavour volatiles are sulphur-containing compounds that occur at low concentrations but have odour detection thresholds that are very low and lipid-derived volatile compounds, which have higher aroma thresholds (Kerth and Miller, 2015). The former are predominant in meat cooked at temperatures greater than 149 °C, whereas the latter predominate in meat cooked at lower temperatures (Dinh *et al.,* 2021; Kerth and Miller, 2015). Therefore, both sulphur-containing flavour precursors (*e.g.*, the amino acids cysteine, cystine, and methionine; the peptide glutathione; and thiamin) and fatty acids are very important (Hou *et al*., 2017).

2.4.2 Tenderness

Tenderness of chicken meat after slaughter, takes less time due to rapid development of rigor mortis. The major factors affecting meat tenderness are the maturity of the connective tissues and contractile state of the myofibril proteins. The maturity of the connective tissue is a function of chemical cross bonding of collagen in the muscles which increases with age (Mir *et al.,* 2017). Probiotics for growth promotion have, however, been postulated to have adverse impact on tenderness development during *post-mortem* storage (Kim *et al.,* 2016). However, higher scores for tenderness have been obtained with thigh meat than breast meat because thigh muscles contain more internal fat and blood capillaries (Melen *et al*., 2014). The physical criteria after slaughter are of importance. Tenderness of meat is a physiological property, that can be influenced by various factors such as breeding, husbandry, feeding, fattening age, slaughter technology, cooling, storing, and not least by the thermic treatment. Tenderness comprises different material properties, like bite characteristics, succulence, and toughness. Meat tenderness originates from 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 (Li *et al.,* 2022).

2.4.3 Juiciness

Juiciness refers mainly to the consumers' perception of juiciness, which is affected by the type of meat (dark *vs.* white broiler meat; the latter has more protein and less fat) and can also depend on primary processing procedures (e.g., water chilling *vs.* air chilling), storage time and conditions, cooking method and preparation. Overall higher moisture and/or fat level increases the perception of juiciness. For example, lean chicken breast fillet (without skin) contains on average 74% moisture, 22% protein and about 2% fat, while chicken thigh meat has 72%, 18% and 8%, respectively (USDA, 2022). This by itself makes breast meat perceived to be juicier when both types of meat are cooked in a similar way. Cooking chicken breast fillets in dry heat (oven) without any marination will result in a dry and chewy product. Therefore, quite a few convenient fresh poultry breast meat products are sold as marinated products (water and spices added; this information must be stated on the label) (Demirok *et al.,* 2013). Furthermore, during cooking meat proteins are denaturated and water is expelled from the meat structure due to both meat shrinkage (less space for water) and lower capacity of the desaturated meat proteins to bind water. This is better seen when dry heat (e.g., conventional oven) is used. Practical solutions for this include marination of whole muscle meat cuts and straight water addition to ground sausage meat batters where the added moisture can compensate for the losses during cooking (Gómez *et al.,* 2019). The ability of meat to retain moisture, with or without marination, also depends on factors such as genetics, body weight, ratio of lean meat to fat, stressors during catching and transportation of live animals to the plant, as well as, chilling method (water *vs.* air as mentioned above) and freezing methods where slow freezing results in large ice crystal formation and more damage to muscle cells compared to fast freezing or individual fast freezing also known as individually quick frozen (IQF) (Mudalal *et al.,* 2015).

2.5 Broiler nutrition and meat quality

Broiler chickens grow faster than any other meat source (Kralik *et al*., 2018), offering good value in terms of animal protein, which has been scientifically proven to be better than plant protein (Berrazaga *et al.,* 2019). In the modern intensive poultry production, feed constitutes up to 70% of the expenditure (Thirumalaisamy *et al.,* 2019). The poultry feed industry is currently facing challenges of volatility in the cost of production due to soaring bills of conventional feedstuffs, leading to the search for alternative feed

ingredients. Cassava by-products are some of the alternatives to replace maize as energy resource in monogastric diets. However, their high fibre, low protein and hydro-cyanide content have limited their use (Latif and Müller, 2015). Cassava stump, which corresponds to the trimmed ends of cassava tubers (Aro *et al.,* 2008), is mostly considered a waste and has not been utilised in the feeding of poultry. Leaf meals have been reported to be good sources of protein. They also have many bioactive compounds that may be useful as alternative to antibiotic growth promoters in monogastric animals. However, these valuable feed resources are also limited by their anti-nutrients and high fibre components. Morgan and Choct (2016) described fungal solid-state fermentation (SSF) as a veritable way of improving the nutritional quality of cassava by-products. Sugiharto *et al.* (2019) reported on the use of enzymes and fermentation to solve the challenges of high fibre and anti-nutritional. factors, thus enhancing the availability of the bioactive compound in leaf meals. In quails, SSF has also improved performance, and carcass quality (Yasar and Gok, 2016). The assessment of meat quality is critical because it focuses on the quality of meat components and the elements that affect palatability. It is also a metric for how long meat can be kept fresh before it starts to spoil (El Masry *et al.,* 2015). Recently, the emphasis has been placed on quality (public health, meat safety, stakeholder competition, and consumer acceptance of production techniques), which is now considered more important than quantity and cost of beef products (Amara *et al*., 2018).

2.6 Conclusions

Broiler chickens play an important source of protein and nutrition to most households globally. The information on the effect of threonine supplementation on productivity and carcass characteristics of indigenous Boschveld chickens is limited and not conclusive. It is, therefore, important to determine the effect of dietary threonine levels on the productivity and carcass characteristics of indigenous Boschveld chickens.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was done at the University of Limpopo Livestock Unit, located at 27.55º south and 24.77º east. The ambient temperature around this area varies from 8 to 22 °C in winter and between 16 and 27 °C in summer. Rainfall averages 450 mm a year (Kutu and Asiwe, 2010).

3.2 Acquisition of resources and chickens housing and management

A total of 275-day-old indigenous Boschveld chicks were bought at Boschveld Ranch, in Bela-Bela, Limpopo Province, South Africa. The ingredients to formulate experimental diets were acquired from Simplegrow Agric Company (Pretoria), Meadow Feeds (Pretoria), and Organics Matter Company (Pretoria), whereas wood shavings, disinfectants, medicines, 175watt infrared ruby lamps, feeders, and drinkers were acquired from NTK and Angel Feeds in Polokwane. An open-sided structure was used to house the chickens. For proper ventilation long axis was situated along an east-west direction in one m^2 pen of wire mesh. Natural (V-shaped windows) and artificial means (supplying fans) were used as ventilation mechanisms to enhance the birds' microclimatic conditions and to maintain the natural convection act. Moreover, the house had temperatures maintained at 30 to 33°C during the starter and 23 to 25°C at the grower phase. Two weeks before the start of the experiment, paraformaldehyde was used to disinfect the poultry house. Lighting was provided 24 hours daily utilizing natural lighting and artificial lighting using 175watt infrared ruby lamps. The bedding for the chickens was prepared using wood shaved sawdust and it was changed weekly. Birds were given fresh feeds and drinking water *ad libitum* hroughout the experiment. Disinfectants were used to wash and cleaned feeders and drinkers daily in the morning before being used. The chicks were vaccinated against infectious bronchitis and Newcastle virus disease using live attenuated virus vaccine (Nobilis® IB 4-91, log10 3, 6 EID50/dose) on day 7, and on days 18 and 28 birds were given live lentogenic virus vaccine (PESTIKAL 1000 dose, Lasota ≥106.0 EID50).

3.3 Experimental diets, designs, and procedures

The first part of the research was to determine the effect of dietary threonine inclusion levels on feed intake, FCR, and body weight of unsexed indigenous Boschveld chickens aged 1 to 49 days. A total of 200-day-old Boschveld chicks, weighing 30 ± 5g per bird, were randomly allocated to 5 diets (dietary treatment levels) in a completely randomized design (CRD), replicated four times with 10 chicks per replicate. The experimental diets were isocaloric and isonitrogenous and were made to fulfil the necessary nutritional standards (NRC, 1994), but with different threonine inclusion levels of 4.0, 7.5, 8.0, 8.5, or 9.0 g per kg DM of diet (Tables 3.01 and 3.02). The chickens were given 20 hours of lighting and 4 hours of darkness from the start to the end of the experiment, to give the chickens time to rest/sleep (NACC, 2010). Water and feeds were supplied *ad libitum* during the experimental period. The experiment was terminated when the chickens were 49 days of age.

The study applied the “three R principle” which are replacement, reduction, and refinement to ensure minimal exposure to any discomfort to chickens (Russell and Burch, 1959). Replacement: refers to technologies or approaches that directly replace or avoid the use of animals; reduction: involves methods that help obtain comparable levels of information from the use of fewer animals and refinement: the optimisation of the methods and the handling of the animals during the experiments and in the husbandry, so that the animals are subjected to as little constraint as possible. The chickens were only handled when necessary (Russell and Burch,1959) to ensure minimal exposure to any discomfort to chickens. Sick chickens and those that refused to eat were isolated and treated accordingly with the help of a veterinarian. The pens were cleaned weekly by the chief researcher, and the chickens were monitored daily to ensure that fresh feed and water were provided *ad libitum*. Rapid weight loss of 15-20% from the normal body weight of chickens during experimental days was frequently monitored. Those with rapid weight loss were isolated and freed from experimental diets and provided with commercial diets. Changes in physical condition such as loss of body weight, abnormal posture; lameness; excessive salivation, heart rate, and respiratory rate were monitored. Any bird showing any of those signs was isolated and treated accordingly. Moreover, external physical appearance (ruffled feathers, etc), behavioural changes (lameness, pecking, aggressive lethargy, fatigue, anorexia, self-

isolating, and hunching, etc), and physiological changes (e.g., body temperature, hormonal fluctuations, clinical pathology) were monitored.

Table 3.01 Dietary treatment codes of Experiment 1 (1-49 days old chickens)

Diet Code	Diet description
ATU_4	Unsexed indigenous Boschveld chickens fed a diet having 4 g of threonine per kg DM.
$ATU_{7.5}$	Unsexed indigenous Boschveld chickens fed a diet having 7.5 g of threonine per kg DM.
ATU_8	Unsexed indigenous Boschveld chickens fed a diet having 8 g of threonine per kg DM.
$ATU_{8.5}$	Unsexed indigenous Boschveld chickens fed a diet having 8.5 g of threonine per kg DM.
ATU_9	Unsexed indigenous Boschveld chickens fed a diet having 9 g of threonine per kg DM.

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

Feed Ingredients	Treatment*				
	ATU_4	**$ATU_{7.5}$**	**ATU_8**	**$ATU_{8.5}$**	**ATU_9**
Yellow maize (%)	60.00	59.02	59.00	58.98	58.96
Maize gluten 60 (%)	1.10	5.00	5.00	5.00	5.00
Wheat bran (%)	3.20	2.50	2.57	2.65	2.71
Soyabean 46 (%)	24.00	24.30	24.20	24.09	24.01
L-lysine (%)	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
Vitamin + 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 phosphate (%)	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)	4.0	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

[†]The ingredients contained in the vitamin–mineral premix were as follows(per kg of diet): vitamin A 12000 IU, vitamin D3 3500 IU, vitamin E30.0 mg, vitamin K 3 2.0 mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine5 mg, vitamin B12 0.02 mg, niacin 50 mg, pantothenate 12 mg, biotin0.01 mg, folic acid 2 mg, Fe 60 mg, Zn 60 mg, Mn 80 mg, Cu 8 mg, Se0.1 mg, Mo 1 mg, Co 0.3 mg, I 1 mg.

The second part of the study was to determine the effect of dietary threonine levels on carcass characteristics and quality of indigenous Boschveld chickens aged 50 to 91 days. Seventy-five male chickens weighing 600 ± 10 g were used in a CRD having 5 treatment groups, replicated 3 times, with 5 chickens per replicate (5 x 3 x 5 = 75 chickens). The experimental diets were formulated to be isocaloric and isonitrogenous and met the nutritional requirements of the chickens (NRC, 1994), but with different threonine inclusion levels of 4.0, 7.5, 8.0, 8.5, or 9.0 g per kg DM of feed (Tables 3.03 and 3.04). The experiment was terminated when the chickens were 90 days of age. The chickens were given 20 hours of lighting and 4 hours of darkness as recommended standard practice for chicken production (NACC, 2010) to ensure adequate growth and development. Water and feeds were supplied *ad libitum* during the experimental period.

Table 3.03 Dietary treatments of the Experiment 2

Diet Code	Diet Description
ATM_4	Male indigenous Boschveld chickens fed a diet having 4 g of threonine per kg DM.
$ATM_{7.5}$	Male indigenous Boschveld chickens fed a diet having 7.5 g of threonine per kg DM.
ATM_8	Male indigenous Boschveld chickens fed a diet having 8 g of threonine per kg DM.
$ATM_{8.5}$	Male indigenous Boschveld chickens fed a diet having 8.5 g of threonine per kg DM.
ATM_9	Male indigenous Boschveld chickens fed a diet having 9 g of threonine per kg DM.

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

Feed Ingredients	**Treatment***				
	ATM_4	**$ATM_{7.5}$**	**ATM_8**	**$ATM_{8.5}$**	**ATM_9**
Yellow maize (%)	60.54	54.00	54.00	54.00	54.00
Maize gluten 60 (%)	1.10	0.37	2.51	2.35	2.18
Wheat bran (%)	3.00	3.24	3.00	3.00	3.00
Soy bean 46 (%)	27.26	30.14	29.02	29.11	29.21
L-lysine (%)	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
Vitamin + minerals premix† (%)	0.10	0.15	0.15	0.15	0.15
Limestone (%)	1.76	1.76	1.77	1.77	1.77
Salt (%)	0.30	0.44	0.44	0.44	0.44
Monocalcium phosphate (%)	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 composition					
Crude protein (%)	21	21	21	21	21
Energy (MJ/kg DM)	14	14	14	14	14
Threonine (g/kg DM)	4.0	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

†The ingredients contained in the vitamin–mineral premix were as follows (per kg of diet): vitamin A 12000 IU, vitamin D3 3500 IU, vitamin E30.0 mg, vitamin K 3 2.0 mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine5 mg, vitamin B12 0.02 mg, niacin 50 mg, pantothenate 12 mg, biotin0.01 mg, folic acid 2 mg, Fe 60 mg, Zn 60 mg, Mn 80 mg, Cu 8 mg, Se0.1 mg, Mo 1 mg, Co 0.3 mg, I 1 mg.

3.4. Data collection

The body weight gain of each chicken was determined at the start of the experiment and thereafter weekly. The BWG of the chickens was determined using their live weights. Daily feed intake was measured by calculating the difference between the total weight of feed offered and the weight of feed leftovers, and the difference was divided by the total number of chickens per pen. The FCR was calculated as the total feed consumed divided by the body weight gain of the birds (McDonald *et al*., 2010).

At 49 and 91 days of age, three chickens from each replicate were sacrificed using the cervical dislocation method, and within 10 seconds after dislocation Thereafter, chicken carcasses were taken to the University of Limpopo Animal Laboratory for further analysis. To ensure that unconsciousness is produced quickly after a cervical dislocation, technical knowledge and skills was required. Cervical dislocation entails holding the legs or wings of the chicken in one hand, and the neck is then extended by tugging on the head and rotating the skull backward (AVMA, 2020).

The chicken's digestive tract was carefully removed from the abdomen without causing tissue damage, and the weight of the organs was measured using an electronic scale sensitive to 0.001 g. The dimension of the small and large intestines was measured using a measuring tape. The pH values of the ileum, crop, gizzard, and caecum digesta were measured with a pH meter. Carcass weights and meat cut-up yields were measured using an electronic weighing scale. Meat pH was measured using the digital pH meter (Crison, Basic 20 pH Meter), while the meat colour was assessed utilizing Hunter-Lab test (L*, a*, b*) system where L* is the lightness, a* is the redness, and b* is the yellowness. Procedures for Warner-Bratzler Shear Force were used to determine the shear force values of the meat (Novaković and Tomašević, 2017).

For sensory attributes, meat samples were frozen at -20 °C and later allowed to be defrosted for 24 hours in a cooler room. The breast meat was cooked in an oven that was preheated to 160 °C. A 1.5 cm thickness of the meat sample was broiled for about 50 minutes, turning it over every 25 minutes. Tongs were used for turning to avoid piercing the meat, which could lead to moisture escaping. The meat was judged on a 5-point scale by a taste panel of 25 assessors for tenderness, juiciness, flavour, and overall acceptability.

The weights of the chicken breast meats before and after roasting were used to determine cooking loss, and the percentage cooking loss was determined as follows:

$$\%\text{Cooking Loss}=\frac{W0-W1}{W0}X\ 100$$

Where W_0 and W_1 are the weights before and after cooking, respectively.

3.5. Chemical analysis

The dry matter (DM) and nitrogen contents of the diets, refusals, faeces, and meat samples were assessed in accordance with the AOAC's guidelines (AOAC, 2012). An adiabatic bomb calorimeter was used to determine the gross energy of the diet and excreta samples. Ion-exchange chromatography was used to analyse the threonine and other amino acid levels of the feeds and meat samples (HPLC, University of Limpopo, South Africa). The calcium and sodium contents were analysed by the methods of AOAC (2012) using the Atomic Absorption Spectrophotometer

(Labtronics). The diets' apparent metabolisable energy (ME) intake using the methods of AOAC (2012).

3.6. Data analysis

Data from the first experiment were subjected to one-way ANOVA using Statistical Analysis System (SAS, 2020) version 9.4 with threonine inclusion level as the main effects. Where there were significant differences ($P<0.05$), the treatment means were separated using a least significant difference (LSD) test at a 5% level of probability (SAS, 2020). The model $Y_{ij} = \mu + T_i + e_{ij}$ was applied where Yij = response variables in diet intake (DM g/bird/day), FCR (g DM feed/g live weight gain), growth and body weight (g/bird/day); μ = constant; Ti = effect of threonine inclusion level; eij = random error.

Data from experiment 2 was subjected to the one-way ANOVA using SAS version 9.4. Where there was a significant F-test ($P<0.05$), the least significance difference (LSD) method was used to separate the means ($P<0.05$) (SAS, 2020).

The model $Y_{ij} = \mu + T_i + e_{ij}$ was utilized.

Where: Y_{ij} = reaction variables in carcass sensory attributes, carcass characteristics, diet intake, FCR, and digestibility, μ = overall mean, T_i = effect of threonine inclusion level, e_{ij} = random error.

The responses in optimal feed intake, live weight, growth rate, FCR, digestibility, and carcass characteristic changes to dietary threonine level were modelled using the following quadratic equation:

$$Y = a + b_1x + b_2x^2 + e$$

Where Y = optimum feed intake, live weight, growth rate, FCR, digestibility, and carcass characteristics; a = intercept; b = coefficients of the quadratic equation; x = dietary threonine level and $-b_1/2b_2$ = x value for optimal response and e = random error. The quadratic model was fitted to the experimental data using the NLIN

procedure of SAS (SAS, 2020). The quadratic model was preferred because it gave the best fit.

The linear relationships between the threonine inclusion levels and reactions in diet intake, FCR, growth rate, live weight, digestibility, and carcass characteristics were determined using the following linear equation:

$$Y = a + bx$$

Where Y = is the FCR, live weight, diet intake, growth, digestibility, carcass characteristics, etc.; b = coefficient of the linear equation; x = the threonine.

CHAPTER FOUR

RESULTS

4.1 Experiment 1

Results of the nutrient composition of the experimental diets are presented in Table 4.01. 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 at starter phase. Thus, any difference in response should be due to dietary threonine level.

Table 4.01 Diet composition (% except MJ/kg DM for energy and g/kg DM for threonine) for Experiment 1

	Treatment#				
Nutrient	**ATU$_4$**	**ATU$_{7.5}$**	**ATU$_8$**	**ATU$_{8.5}$**	**ATU$_9$**
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	4	7.5	8	8.5	9

ATU: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

Results of the effect of threonine inclusion levels on feed intake (g DM/bird/day), body weight gain (g/bird/day), and FCR (g feed intake/g body weight gain) of unsexed indigenous Boschveld chickens aged 1-49 days are presented in Table 4.02. Threonine inclusion levels had effect ($p<0.05$) on feed intake, body weight gain (BWG), and FCR of unsexed indigenous Boschveld chickens aged 1-49 days.

Unsexed indigenous Boschveld chickens on a diet having 8 g of threonine per kg DM had higher ($p<0.05$) feed intake than those on diets containing 4, 7.5, and 8.5 of threonine per kg DM. Similarly, unsexed chickens fed a diet having 4 g of threonine per kg DM had higher ($p<0.05$) feed intake than those on diets having 7.5 and 8.5 g of threonine/kg DM. However, chickens fed diets containing 8 and 9 g of threonine per kg DM had similar ($p>0.05$) feed intakes.

Unsexed indigenous Boschveld chickens on a diet containing 9 g of threonine per kg DM had higher ($p<0.05$) BWG than those on diets having 4, 7.5, and 8.5 g of threonine per kg DM. Similarly, chickens fed a diet having 8.5 g of threonine per kg DM had higher ($p<0.05$) BWG than those on diets having 4 and 7.5 g of threonine/kg DM. However, unsexed chickens fed diets having 8 and 9 g of threonine per kg DM had similar ($p>0.05$) BWG.

Unsexed indigenous Boschveld chickens on a diet having 8.5 g of threonine per kg DM had lower ($p<0.05$) FCR values than those on diets having 4, 7.5, 8 and 9 g of threonine/kg DM. Similarly, unsexed chickens fed a diet having 4 g of threonine per kg DM had higher ($p>0.05$) FCR values than those on diets having 7.5, 8, 8.5 and 9 g of threonine/kg DM. However, unsexed chickens fed diets having 8 and 9 g of threonine per kg DM had similar ($p>0.05$) FCR values.

Table 40.2 Effect of threonine inclusion levels on feed intake (g DM/bird/day), body weight gain (g/bird/day), and feed conversion ratio (g feed intake/g body weight gain) of unsexed indigenous Boschveld chickens aged 1-49 days.

Variable	Treatment					P-Value
	ATU_4	$ATU_{7.5}$	ATU_8	$ATU_{8.5}$	ATU_9	
FI	25.23^{b}± 0.046	22.71^{c}± 0.040	25.78^{a}± 0.023	20.05^{d}± 0.015	25.74^{a}± 0.009	<.0001
BWG	10.49^{d}± 0.046	10.84^{c}± 0.012	12.08^{a}± 0.023	11.24^{b}± 0.015	12.10^{a}±0.012	<.0001
FCR	2.41^{a}±0.006	2.10^{c}±0.003	2.13^{b}±0.006	1.78^{d}±0.000	2.13^{b}±0.003	<.0001

a, b, c : Means in the same row not sharing a common superscript are significantly different (P<0.05)

ATU: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

Variables: Values presented as mean ± standard error.

A positive relationship was observed between threonine inclusion level and BWG (g/bird/day) (r^2 = 0.583) in unsexed indigenous Boschveld chickens aged 1-49 days (Figures 4.02 and Table 4.03). However, a negative relationship was observed between threonine inclusion level and feed intake (g DM/bird/day) (r^2 = 0.059) of unsexed indigenous Boschveld chickens aged 1-49 days (Figures 4.01 and Table 4.03).

Table 4.03 Relationships between threonine inclusion level and feed intake (g DM/bird/day) and BWG (g/bird/day) of unsexed indigenous Boschveld chickens aged 1-49 days

Factor	**Formula**	**r^2**	**Probability**
Feed intake	Y = -0.31x + 26.17	0.059	0.694
Body weight gain	Y = 0.28x + 9.28	0.583	0.133

r^2: Coefficient of determination

P: Probability-value

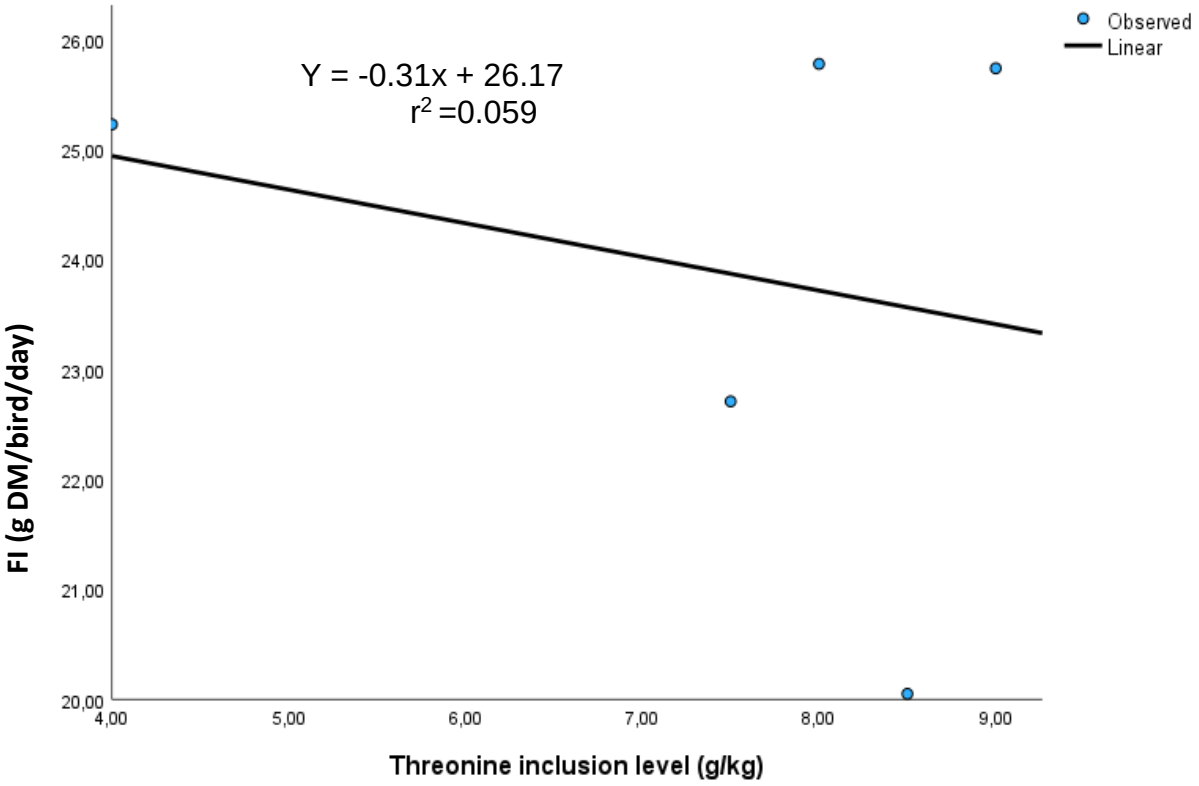

Figure 4.01 The relationship between threonine inclusion level and feed intake (g DM/bird/day) of unsexed indigenous Boschveld chickens aged 1-49 days

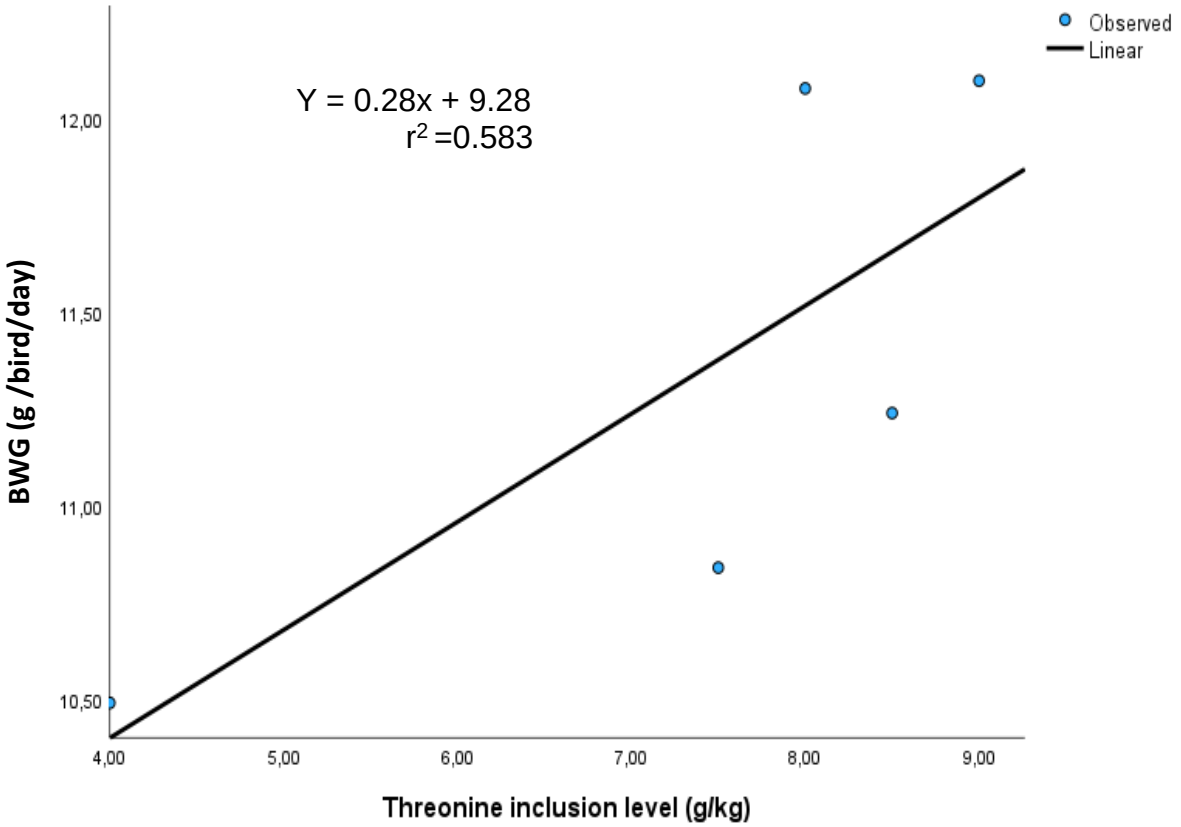

Figure 4.02 The relationship between threonine inclusion level and body weight gain (g/bird/day) of unsexed indigenous Boschveld chickens aged 1-49 days

Results on the effect of threonine inclusion levels of on gut organ pH, length (cm) and weight (g) of unsexed indigenous Boschveld chickens aged 49 days are presented in Table 4.04. Threonine inclusion levels had no effect ($p>0.05$) crop, gizzard, ileum, jejunum, caeca, and large intestine pH of unsexed indigenous Boschveld chickens. However, threonine inclusion levels had effect ($p<0.05$) on duodenum pH of unsexed indigenous Boschveld chickens aged 49 days. Unsexed indigenous Boschveld chickens on diets containing 8 g of threonine per kg DM had higher ($p<0.05$) duodenum pH value than those on diets 4, 7.5, 8.5 and 9 g of threonine per kg DM. Unsexed indigenous Boschveld chickens on diets containing 7.5 or 8 g of threonine per kg DM had higher ($p<0.05$) duodenum pH value than those on a diet containing 4 g of threonine per kg DM. However, unsexed chickens fed diets having 7.5, 8, 8.5 and 9 g of threonine per kg of DM had similar ($p>0.05$) duodenum pH value. Similarly, unsexed chickens fed diets having 8.5 and 9 g of threonine/kg DM had similar ($p>0.05$) duodenum pH values.

Table 4.04 Effect of threonine inclusion levels on gut organ pH, length (cm) and weight (g) of unsexed indigenous Boschveld chickens aged 49 days

Variables	Treatment					P-Value
	ATU_4	$ATU_{7.5}$	ATU_8	$ATU_{8.5}$	ATU_9	
Gut organ pH						
Crop	5.64±0.119	5.63±0.099	5.59±0.095	5.55±0.113	5.55±0.068	0.9434
Gizzard	5.74±0.072	5.68± 0.079	5.76±0.015	5.73±0.023	5.66±0.009	0.6337
Ileum	5.79±0.111	5.56±0.074	5.63±0.034	5.58±0.147	5.60±0.039	0.4616
Jejunum	5.35±0.091	5.46±0.012	5.50±0.023	5.39±0.007	5.47±0.066	0.3182
Ddm	5.43^{b}±0.033	5.59^{a}±0.026	5.69^{a}±0.023	5.54^{ab}±0.027	5.49^{ab}±0.015	0.0039
Caeca	5.63±0.055	5.68±0.047	5.65±0.139	5.74±0.003	5.57±0.064	0.2583
LI	5.64±0.018	5.81±0.100	5.67±0.117	5.70±0.013	5.65±0.066	0.5305
Gut organ length (cm)						
GIT	109.20^{a}±2.425	95.06^{b}±2.742	95.50^{b}±0.173	102.60^{ab}±1.270	101.30^{ab}±0.863	0.0012
Ileum	42.37^{a}±1.179	36.17^{b}±0.088	3703^{b}±0.797	41.27^{a}±0.433	39.17^{ab}±0.433	0.0003
Jejunum	46.90^{a}±1.266	35.30^{c}±1.328	38.10^{c}±0.971	43.80^{ab}±1.732	39.20^{bc}±0.404	0.0004
Ddm	21.30^{a}±1.270	20.00^{ab}±0.692	14.80^{c}±0.173	17.60^{bc}±0.000	18.37^{ab}±0.033	0.0004
Caeca	23.33±1.519	24.40±0.982	22.27±0.549	22.97±0.895	24.20±0.115	0.5062
LI	5.23^{ab}±0.202	5.37^{ab}±0.033	4.90^{b}±0.058	5.60^{a}±0.058	5.57^{a}±0.260	0.0501
Gut organ weight (g)						
Crop	7.50^{ab}±0.289	4.50^{b}±0.346	6.17^{ab}±0.797	5.37^{b}±0.202	5.37^{b}±0.260	0.0068
Gizzard	34.67^{a}±2.223	25.17^{b}±2.194	23.13^{b}±1.353	25.27^{b}±1.299	26.87^{b}±0.722	0.0052
Ileum	9.10^{a}±0.115	8.80^{a}±0.115	6.27^{c}±0.033	6.20^{c}±0.404	7.87^{b}±0.033	<.0001
Jejunum	11.17^{ab}±0.145	10.30^{bc}±0.115	8.77^{c}±0.033	12.40^{a}±0.173	9.67^{bc}±0.780	0.0004
Ddm	5.07^{b}±0.491	6.07^{ab}±0.033	5.27^{b}±0.203	6.77^{a}±0.088	5.50^{b}±0.231	0.0063
Caeca	5.40^{a}±0.058	3.60^{c}±0.173	3.77^{c}±0.033	4.80^{b}±0.058	5.10^{b}±0.058	<.0001
LI	1.53±0.208	1.30±0.100	1.13±0.153	1.40±0.346	1.30±0.100	0.2555

a, b, c, : Means in the same row not sharing a common superscript are significantly different (P<0.05)

ATU: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

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

P value: Standard error of the means.

Threonine inclusion levels had effect ($p<0.05$) on GIT, ileum, jejunum, duodenum, and large intestine lengths of unsexed indigenous Boschveld chickens aged 49 days. However, threonine inclusion levels had no effect ($p>0.05$) on caeca lengths of indigenous Boschveld chickens aged 49 days.

Unsexed indigenous Boschveld chickens on diets containing 4 g of threonine per kg DM had longer ($p<0.05$) GIT lengths than those on diets containing 7.5 and 8 g of threonine per kg DM. However, unsexed chickens fed diets having 4, 8.5 and 9 g of threonine per kg of DM had similar ($p>0.05$) GIT lengths. Similarly, unsexed chickens fed diets having 8.5 and 9 g of threonine/kg DM had similar ($p>0.05$) GIT lengths.

Unsexed indigenous Boschveld chickens on diets containing 4 g of threonine per kg DM had longer ($p<0.05$) ileum lengths than those on diets containing 7.5 and 8 g of threonine per kg DM. However, unsexed chickens fed diets having 4, 8.5 and 9 g of threonine per kg of DM had similar ($p>0.05$) ileum lengths. Similarly, unsexed chickens fed diets having 7.5 and 8 g of threonine/kg DM had similar ($p>0.05$) ileum lengths.

Unsexed indigenous Boschveld chickens fed diets containing 4 g and 8.5 gthreonine/kg DM had longer ($p<0.05$) jejunum than other dietary treatments. Unsexed indigenous Boschveld chickens on diets containing 8.5 g of threonine/kg DM had longer ($p<0.05$) jejunum lengths than their counterparts on diets containing 9 g threonine per kg DM. However, unsexed chickens fed diets having 4 and 9 g of threonine per kg of DM had similar ($p>0.05$) jejunum lengths.

Unsexed indigenous Boschveld chickens on diets containing 4 g, 7.5 g and 9.5 g threonine per kg DM had similar lengths. The duodenum length for chickens on diets containing 8 and 8.5 g threonine per kg DM were also similar. Unsexed indigenous Unsexed Boschveld chickens on diets containing 4 g threonine per DM had longer duodenum than those on 8 and 8.5 g threonine per kg DM. However, duodenum lengths for birds on diets containing 4, 7.5 and 9 g threonine per kg DM were not significantly ($p>0.05$) from each other. The duodenum lengths for birds on 7.5, 8.5 and 9 g threonine per kg DM were similar. Similarly, duodenum lengths of unsexed Boschveld chickens fed diets containing 7.5, 8.5 and 9 g threonine per kg DM were not significantly ($p>0.05$) different from each other.However, unsexed chickens fed

diets having 7.5 and 8 g of threonine per kg of DM had similar ($p>0.05$) duodenum lengths.

As shown in Table 4.04, dietary treatment levels had no effect ($p>0.05$) on the length of caeca. Boschveld chickens fed diets containing 8 g threonine per kg DM had statistically ($p<0.05$) shorter large intestines compared to those fed diets containing 8.5 and 9 g threonine per kg DM. On the other hand, chickens fed diets containing 4. 7.5, 8.5 and 9 g threonine per kg DM had similar ($p>0.05$) large intestine length. Similarly, the length of large intestine for birds on diets containing 4, 7.5 and 8 g threonine per kg DM was not significantly different ($p>0.05$) from each other.

Threonine inclusion levels had no effect ($p>0.05$) on large intestine weights of indigenous Boschveld chickens aged 49 days. However, threonine inclusion levels had effect ($p<0.05$) on crop, gizzard, ileum jejunum, duodenum, and caeca weights of indigenous Boschveld chickens aged 49 days. Unsexed indigenous Boschveld chickens on diets containing 4 and 8 g threonine per kg DM had heavier ($p<0.05$) crop weights than those on a diet containing 7.5, 8.5 and 9 g of threonine per kg DM. However, unsexed chickens fed diets having 7.5, 8.5 and 9 g of threonine per kg of DM had similar ($p>0.05$) crop weights. Similarly, unsexed chickens fed diets having 4 and 8 g of threonine/kg DM had the same ($p>0.05$) crop weights. Unsexed indigenous Boschveld chickens on a diet containing 4 g of threonine per kg DM had heavier ($p<0.05$) gizzard weights than those on diets containing 7.5, 8, 8.5 and 9 g of threonine per kg DM. However, unsexed chickens fed diets having 7.5, 8, 8.5, and 9 g of threonine per kg of DM had similar ($p>0.05$) gizzard weight.

Unsexed indigenous Boschveld chickens on diets containing 4 and 7.5 g of threonine/kg DM had heavier ($p<0.05$) ileum weights than other dietary treatments. On the other hand, diets containing 8 and 8.5 g threonine had shorter ileum than other dietary treatments. However, unsexed chickens fed diets having 8 and8.5 gthreonine per kg of DM had similar ($p>0.05$) ileum weights. Also, unsexed chickens fed diets having 4, and 7.5 g threonine/kg DM had similar ($p>0.05$) ileum weight. Please say something about 9 g threonine in relation to other treatments,

According to Table 4.04, chickens on diets containing 8 and 8.5 g threonine per kg DM had lower and heavier jejunum weights compared to other dietary treatments. unsexed However, jejunum weight for chickens fed diets containing 4 and 8.5 g threonine per kg DM were not statisticaly different ($p>0.05$). Ileum weight for birds on 7.5 and 8 g threonine were similar ($p>0.05$). Furthermore, unsexed indigenous Boschveld chickens on diets containing 8.5 and 9 g of threonine/kg DM had similar ($p>0.05$) jejunum weight. No statistical difference ($p>0.05$) could be found for birds on diets containing 4, 7.5 and 9 g threonine/ kg DM. Furthermore, the jejunum weight for birds on 7.5, 8 and 9 g threonine/kg DM was similar.

Unsexed indigenous Boschveld chickens on diets containing 8.5 g threonine/kg DM had heavier ($p<0.05$) duodenum weights which was not statistically different ($p>0.05$) for birds fed diets containing 7.5 g threonine. The weight duodenum of chickens fed diets having 4, 7.5, 8.0 and 9 g of threonine per kg DM was similar ($p>0.05$).

Unsexed indigenous Boschveld chickens on diets containing 4 g of threonine/kg DM had heavier ($p<0.05$) caeca weights compared to other dietary treatments. The weight of caeca for chickens fed diets containing 7.5 and 8 g threonine were similar ($p>0.05$). No significant difference in the weight of caeca was observed in birds fed diets containing 8.5 and 8 g threionine per kg DM.

Duodenum pH and duodenum weight were optimized (r^2 = 0.753 and 0.300) at threonine inclusion level of 0.70 and 8.00 for unsexed Boschveld chickens aged 49 days (Figures 4.03, 4.13 and Table 4.05, respectively). A positive relationship was observed between threonine inclusion level and large intestine lengths (r^2 = 0.115) of unsexed indigenous Boschveld chickens aged 49 days (Figure 4.09 and Table 4.06). A negative relationship was observed between threonine inclusion level and GIT, ileum, jejunum, and duodenum lengths (r^2 = 0.411, 0.233, 0.363 and 0.424 respectively) in unsexed indigenous Boschveld chickens aged 1-49 days (Figures 4.04, 4.05, 4.06, 4.07, respectively and Table 4.06).

A negative relationship was observed between threonine inclusion level and crop, gizzard, ileum, jejunum, and caeca weights (r^2 = 0.588, 0.749, 0.404, 0.054 and 0.130

respectively) of unsexed Boschveld chickens aged49 days (Figures 4.09, 4.10, 4.11, 4.12 and 4.14, respectively and Table 4.06).

Table 4.05 Threonine inclusion levels for optimal gut organ characteristics of unsexed indigenous Boschveld chickens aged 49 days

Factor	Formula	X	Y	r^2	P
Duodenum pH	$Y = 4.24 + 0.42x + -0.03x^2$	0.70	4.52	0.753	0.247
Duodenum (g)	$Y = 2.21 + 0.96x + -0.06x^2$	8.00	6.05	0.300	0.700

X: Inclusion level for optimal value

Y: Optimal Y-level

r^2: Coefficient of determination

P: Probability-value

Table 4.06 Relationships between threonine inclusion level and gut organ lengths and weights of unsexed indigenous Boschveld chickens aged 49 days

Factor	Formula	r^2	Probability
GIT (cm)	Y = -1.88x + 114,65	0.411	0.243
Ileum (cm)	Y = -0.65x + 43.99	0.233	0.411
Jejunum (cm)	Y = -1.41x + 51.11	0.363	0.282
Duodenum (cm)	Y = -0.82x + 24.44	0.424	0.234
LI (cm)	Y = 0.05x + 4.97	0.115	0.577
Crop (g)	Y = -0.44x + 9.01	0.588	0.131
Gizzard (g)	Y = -1.96x + 41.49	0.749	0.058
Ileum (g)	Y = -0.44x + 10.89	0.404	0.249
Jejunum (g)	Y = -0.16x + 11.68	0.054	0.706
Caeca (g)	Y = -0.15x + 5.62	0.130	0.552

r^2: Coefficient of determination

P: Probability-value

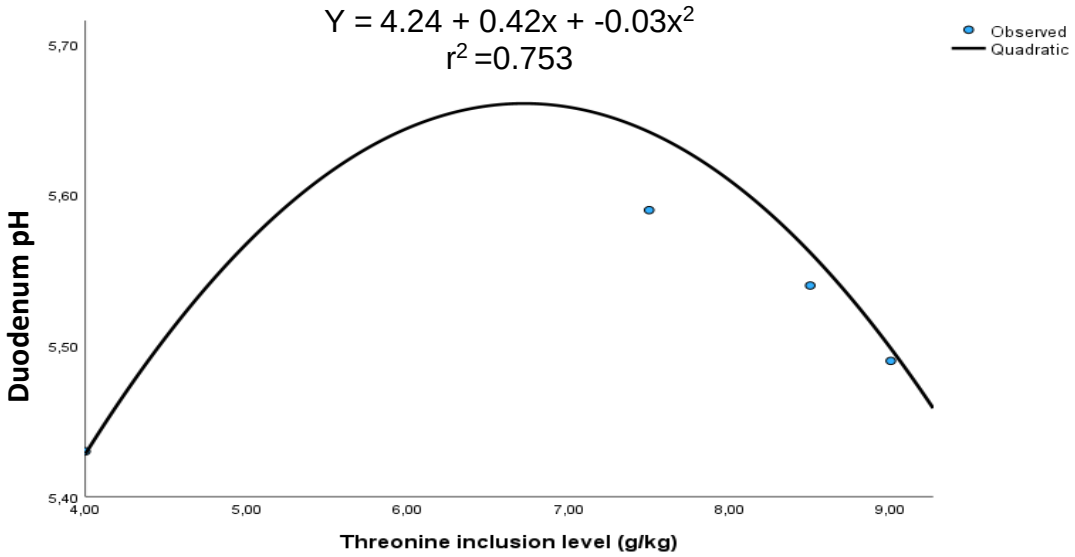

Figure 4.03 Effect of threonine inclusion level on duodenum pH of unsexed indigenous Boschveld chickens aged 49 days

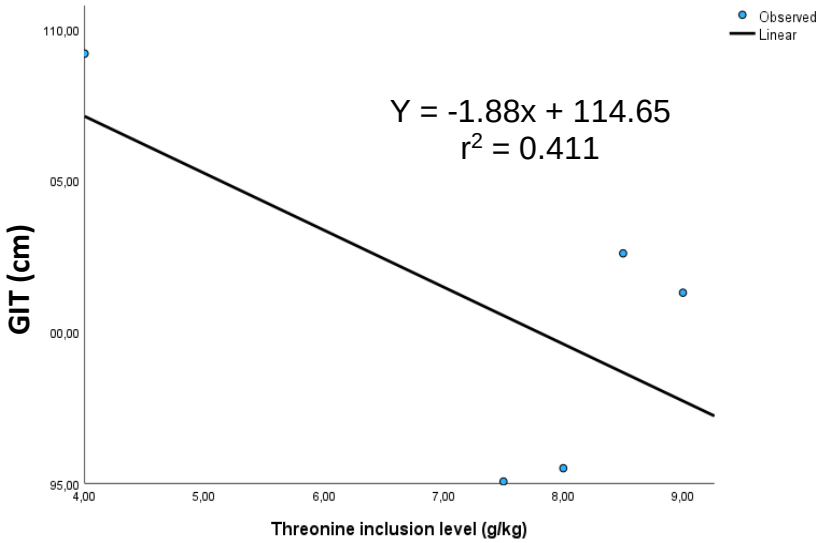

Figure 4.04 Relationship between threonine inclusion level and GIT lengths of unsexed indigenous Boschveld chickens aged 49 days

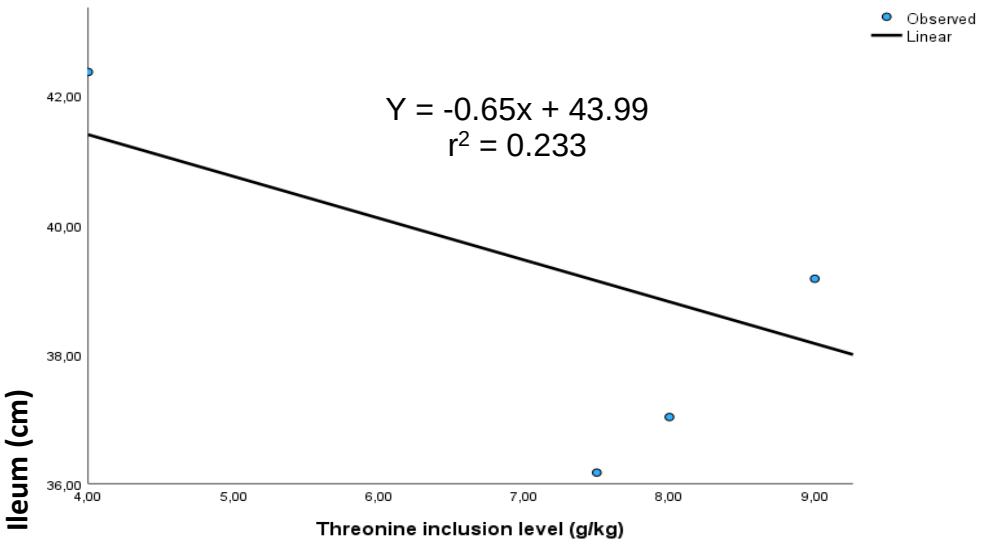

Figure 4.05 Relationship between threonine inclusion level and ileum lengths of nsexed indigenous Boschveld chickens aged 49 days

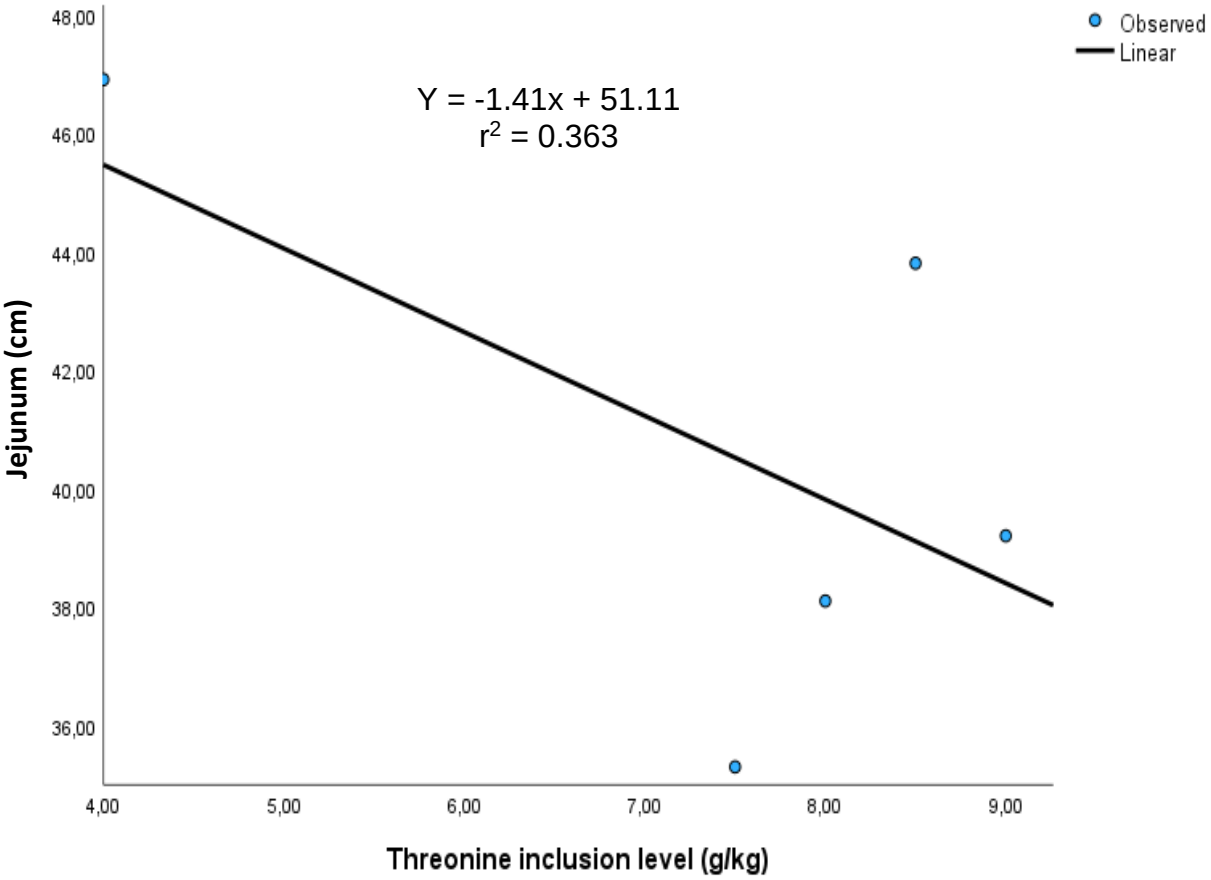

Figure 4.06 Relationship between threonine inclusion level and jejunum lengths of unsexed indigenous Boschveld chickens aged 49 days

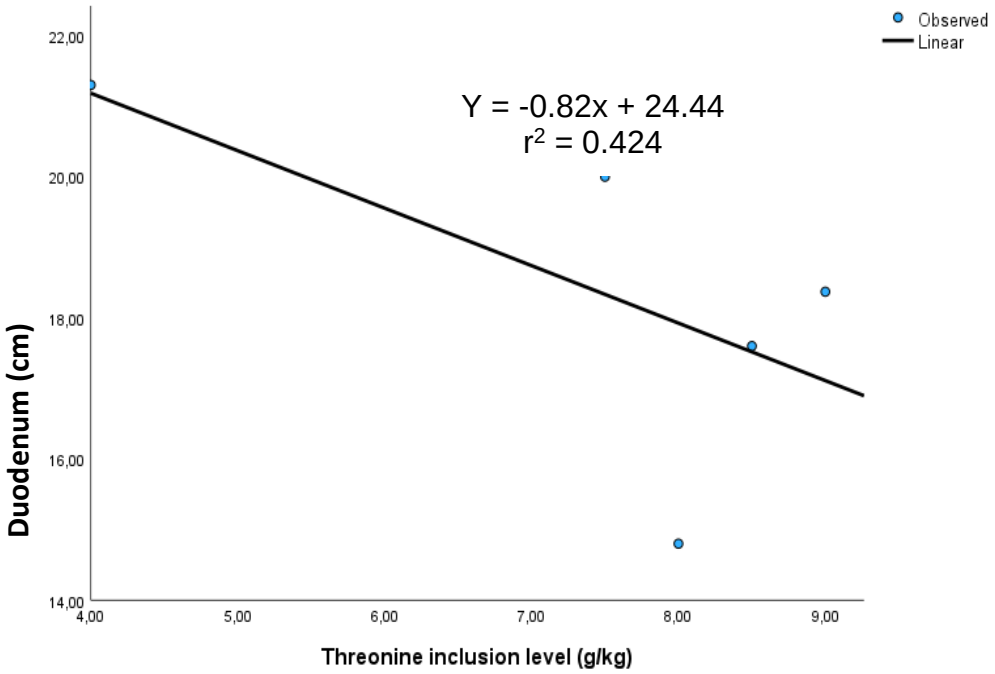

Figure 4.07 Relationship between threonine inclusion level and duodenum lengths of unsexed indigenous Boschveld chickens aged 49 days

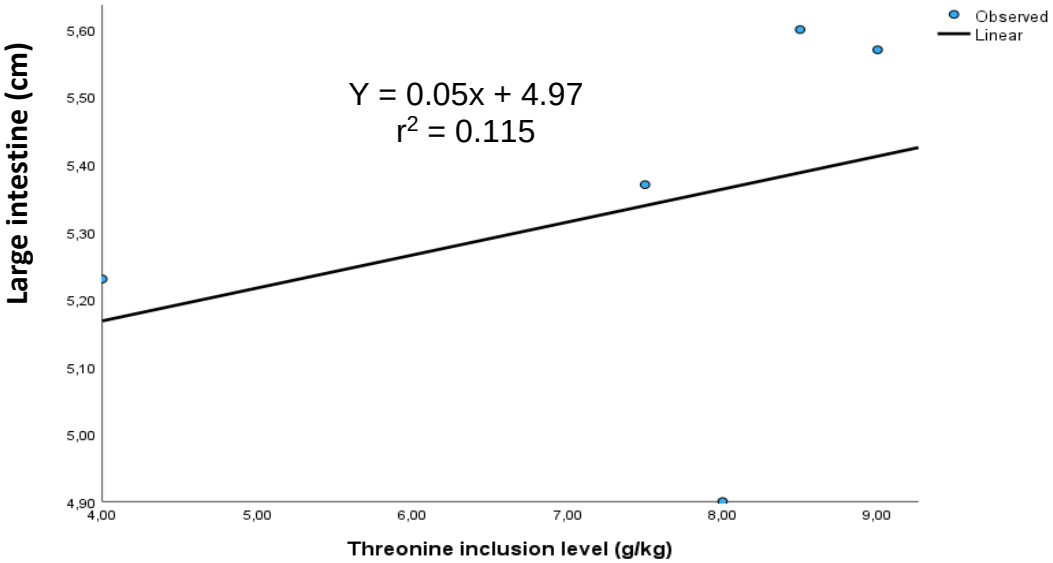

Figure 4.08 Relationship between threonine inclusion level and large intestine lengths of unsexed indigenous Boschveld chickens aged 49 days

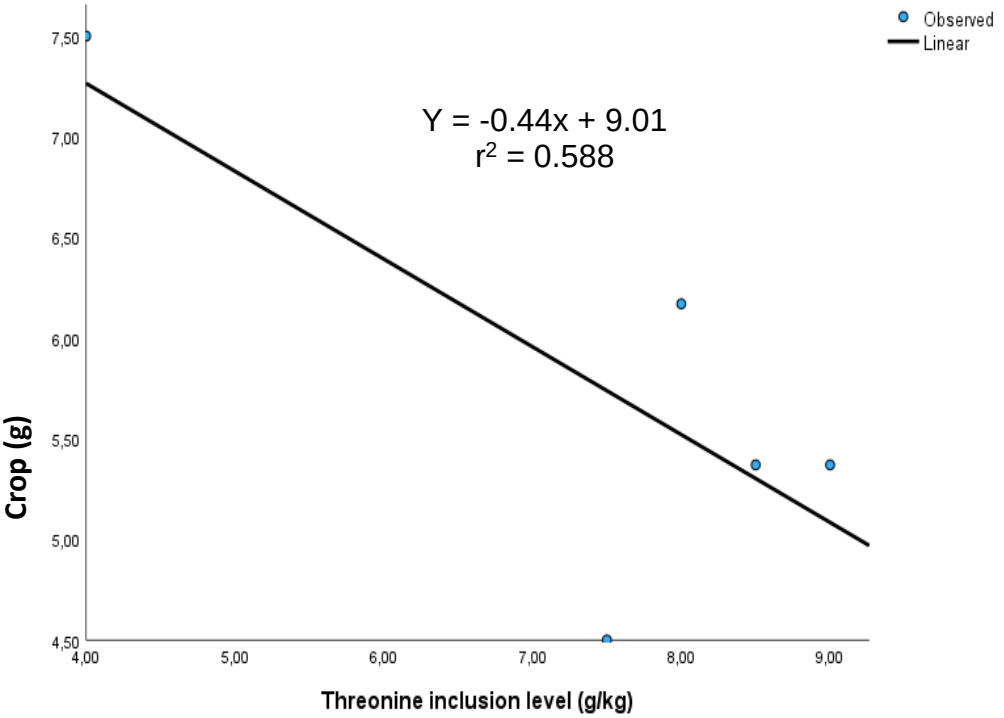

Figure 4.09 Relationship between threonine inclusion level and crop weights of unsexed indigenous Boschveld chickens aged 49 days

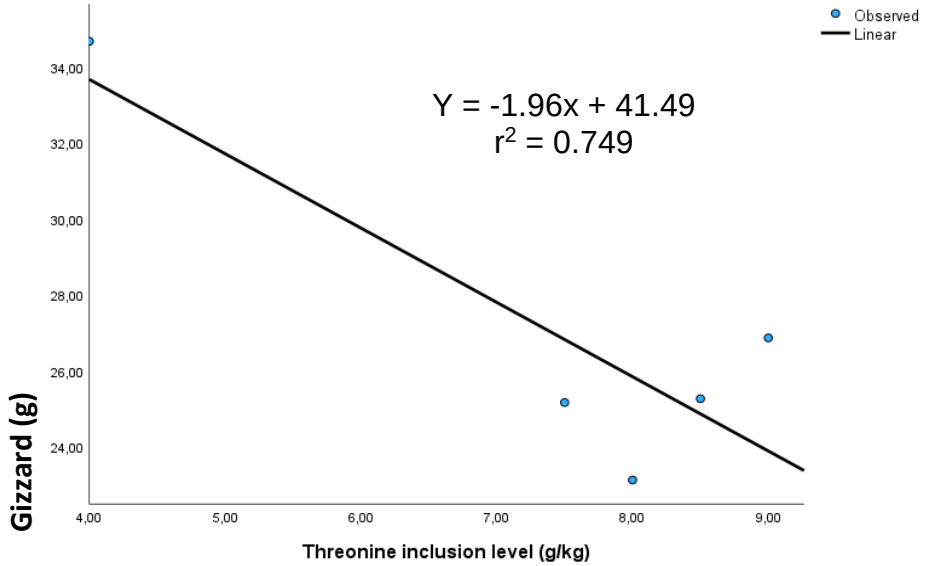

Figure 4.10 Relationship between threonine inclusion level and gizzard weights of unsexed indigenous Boschveld chickens aged 49 days

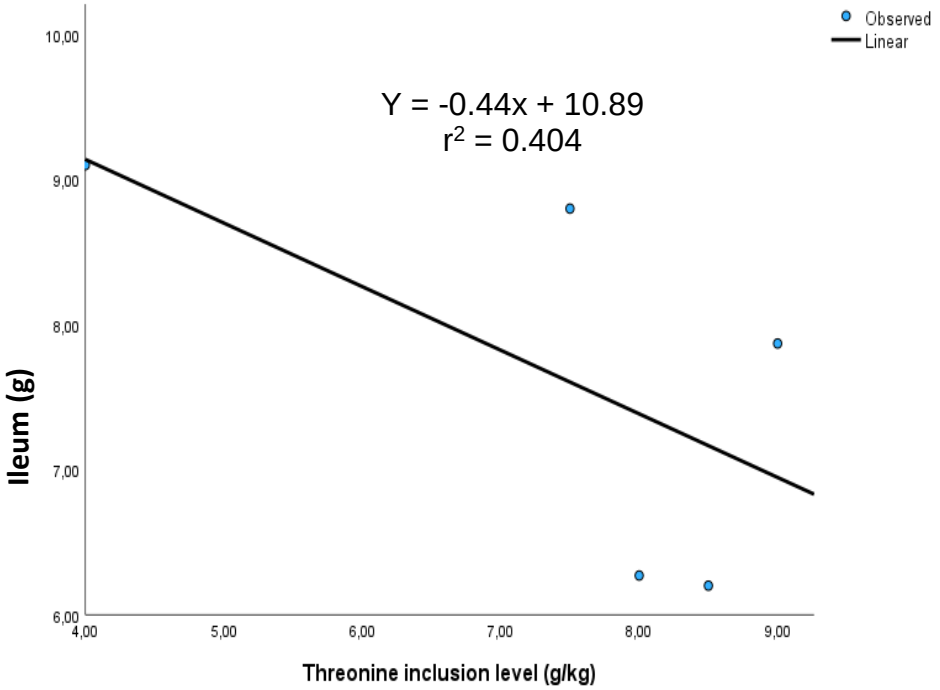

Figure 4.11 Relationship between threonine inclusion level and ileum weights of unsexed indigenous Boschveld chickens aged 49 days

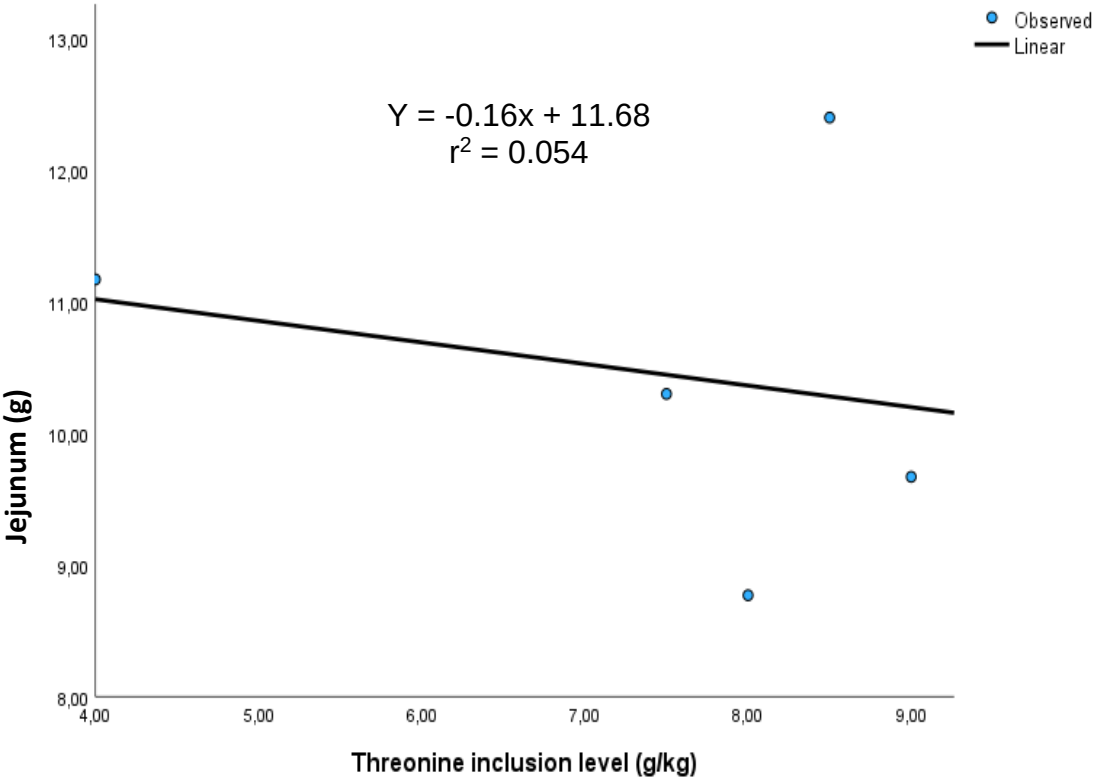

Figure 4.12 Relationship between threonine inclusion level and jejunum weights of unsexed indigenous Boschveld chickens aged 49 days

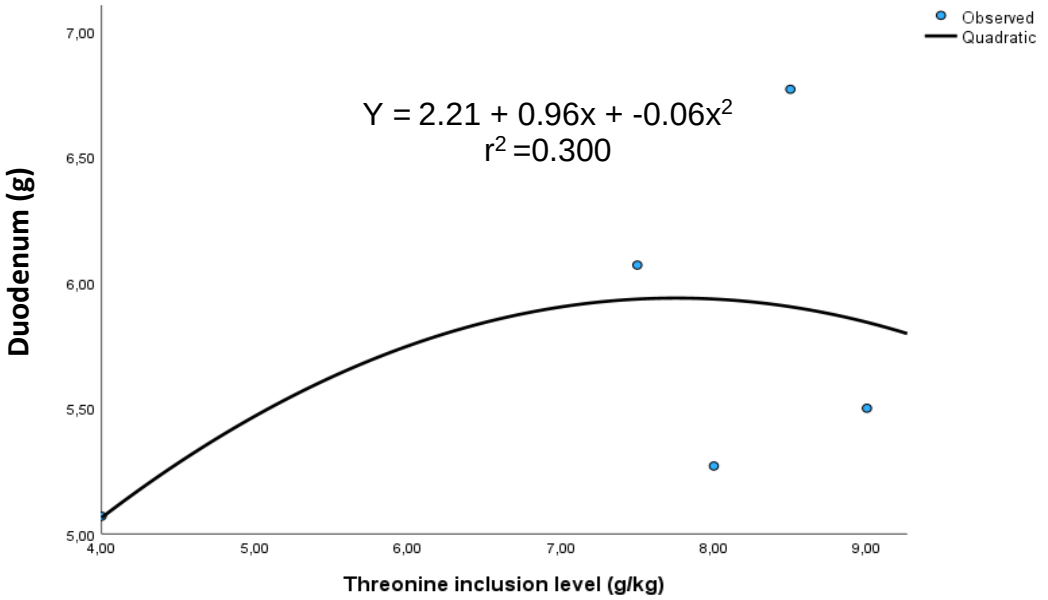

Figure 4.13 The effect of threonine inclusion level on duodenum weights of unsexed indigenous Boschveld chickens aged 49 days

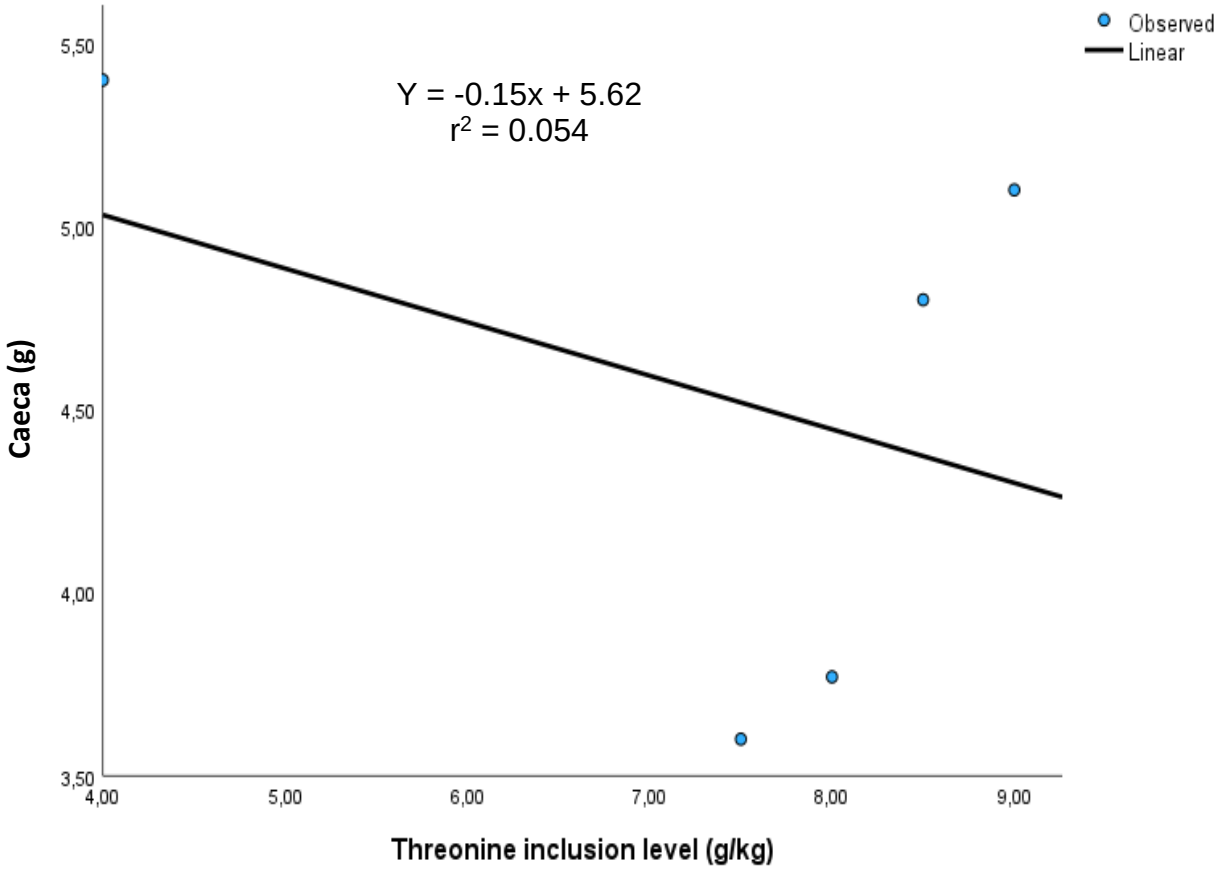

Figure 4.14 Relationship between threonine inclusion level and caeca weights of unsexed indigenous Boschveld chickens aged 49 days.

4.2 Experiment 2

Results of the nutrient composition of the experimental diets are presented in Table 4.07. 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 indigenous chickens in grower phase. Thus, any difference in response should be due to dietary threonine level.

Table 4.07 Diet composition (% except MJ/kg DM for energy and g/kg DM for threonine) for Experiment 2

	Treatment#				
Nutrient	**ATM4**	**ATM7.5**	**ATM8**	**ATM8.5**	**ATM9**
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	4	7.5	8	8.5	9

ATM: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

Results on the effect of threonine inclusion levels on feed intakes (g DM/bird/day), BWGs (g/bird/day), and FCR (g feed intake/g BWG) of male indigenous Boschveld chickens aged 50-91 days are presented in Table 4.08. Threonine inclusion levels had the effect ($p<0.05$) on feed intakes, BWG, and FCR of male indigenous Boschveld chickens aged 50-91 days.

Male indigenous Boschveld chickens on a diet having 4, 8 and 8.5 g threonine per kg DM had higher ($p<0.05$) feed intakes than those on diets containing 7.5 or 9 g of threonine per kg DM. However, chickens fed diets containing 7.5 and 9 g of threonine per kg DM had similar ($p>0.05$) feed intakes. Furthermore, chickens fed diets containing 4, 8 and 8.5 g of threonine per kg DM had similar ($p>0.05$) feed intakes.

Male indigenous Boschveld chickens on a diet containing 4, 7.5, 8 and 8.5 g of threonine per kg DM had heavier ($p<0.05$) BWGs than those fed diets having 9 g of threonine per kg DM. However, male chickens fed diets containing 4, 7.5, 8 or 8.5 g of threonine per kg DM had similar ($p>0.05$) BWGs.

Male indigenous Boschveld chickens on a diet having 7.5 g of threonine per kg DM had lower ($p<0.05$) FCR values than those on diets having 7.5, 8, 8.5 and 9 g of threonine/kg DM. Similarly, male chickens fed a diet having 4 g of threonine per kg DM had higher ($p>0.05$) FCR values than those on diets having 7.5, 8, 8.5 and 9 g of threonine/kg DM. However, unsexed chickens fed diets having 4, 8 and 8.5 g of threonine per kg DM had similar ($p>0.05$) FCR values.

Body weight gain (g/bird/day) and FCR (g feed intake/g BWG of male indigenous Boschveld chickens aged 91 days were optimized (r^2= 0.366, 0.479) at threonine inclusion levels of 6.12 and 6.82 f(Figures 4.16, 4.17, respectively and Table 4.09). The negative relationship was observed between threonine inclusion level and feed intake (g DM/bird/day) (r^2 = 0.281) of male indigenous Boschveld chickens aged 50-91 days (Figure 4.15 and Table 4.09).

Table 4.08 Effect of threonine inclusion levels on feed intake (g DM/bird/day), body weight gain (g/bird/day), and feed conversion ratio (g feed intake/g body weight gain) of male indigenous Boschveld chickens aged 50-91 days

Variable	Treatment					P-Value
	ATM_4	$ATM_{7.5}$	ATM_8	$ATM_{8.5}$	ATM_9	
FI	140.98^{a}±0.995	111.72^{b}±0.104	135.62^{a}±3.605	136.26^{a}±2.074	105.88^{b}±2.315	<.0001
BWG	22.75^{a}±0.251	23.43^{a}±0.485	23.46^{a}±0.098	23.70^{a}±0.346	19.77^{b}±0.101	<.0001
FCR	6.20^{a}±0.095	4.77^{c}±0.095	5.78^{ab}±0.175	5.75^{ab}±0.171	5.35^{bc}±0.094	0.0002

a, b, c : Means in the same row not sharing a common superscript are significantly different (P<0.05)

ATM: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed).

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

Table 4.09 Threonine inclusion levels for optimal feed intake (g DM/bird/day) and feed conversion ratio (g feed intake/g body weight gain) of male Indigenous Boschveld chickens aged 50-91 days

Factor	Formula	X	Y	r^2	P
BWG	$Y = 4.56 + 6.73x + -0.55x^2$	6.12	25.15	0.634	0.366
FCR	$Y = 10.47 + -1.50x + 0.11x^2$	6.82	5.36	0.479	0.521

X: Inclusion level for optimal value

Y: Optimal Y-level

r^2: Coefficient of determination

P: Probability-value

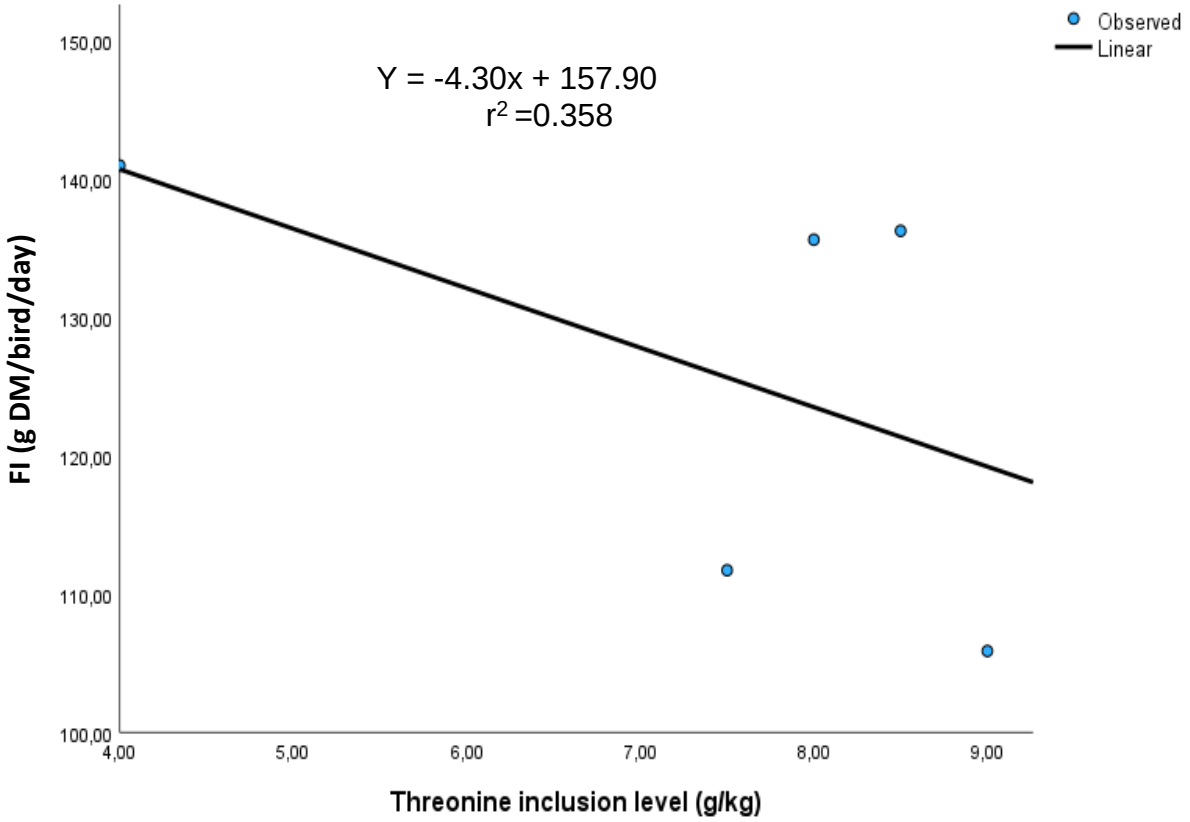

Figure 4.15 The relationship between threonine inclusion level and feed intake (g DM/bird/day) of male indigenous Boschveld chickens aged 50-91 days

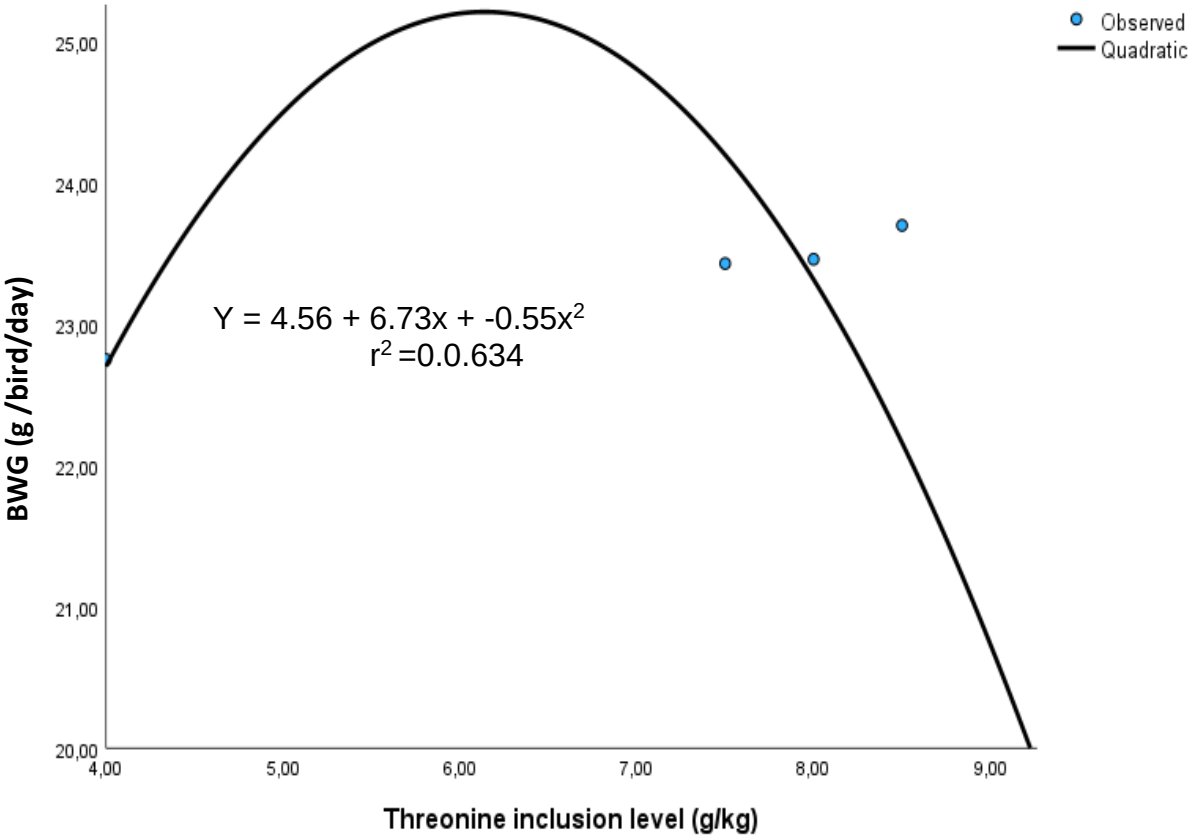

Figure 4.16 Effect of threonine inclusion level on body weight gain of male indigenous Boschveld chickens aged 50-91 days

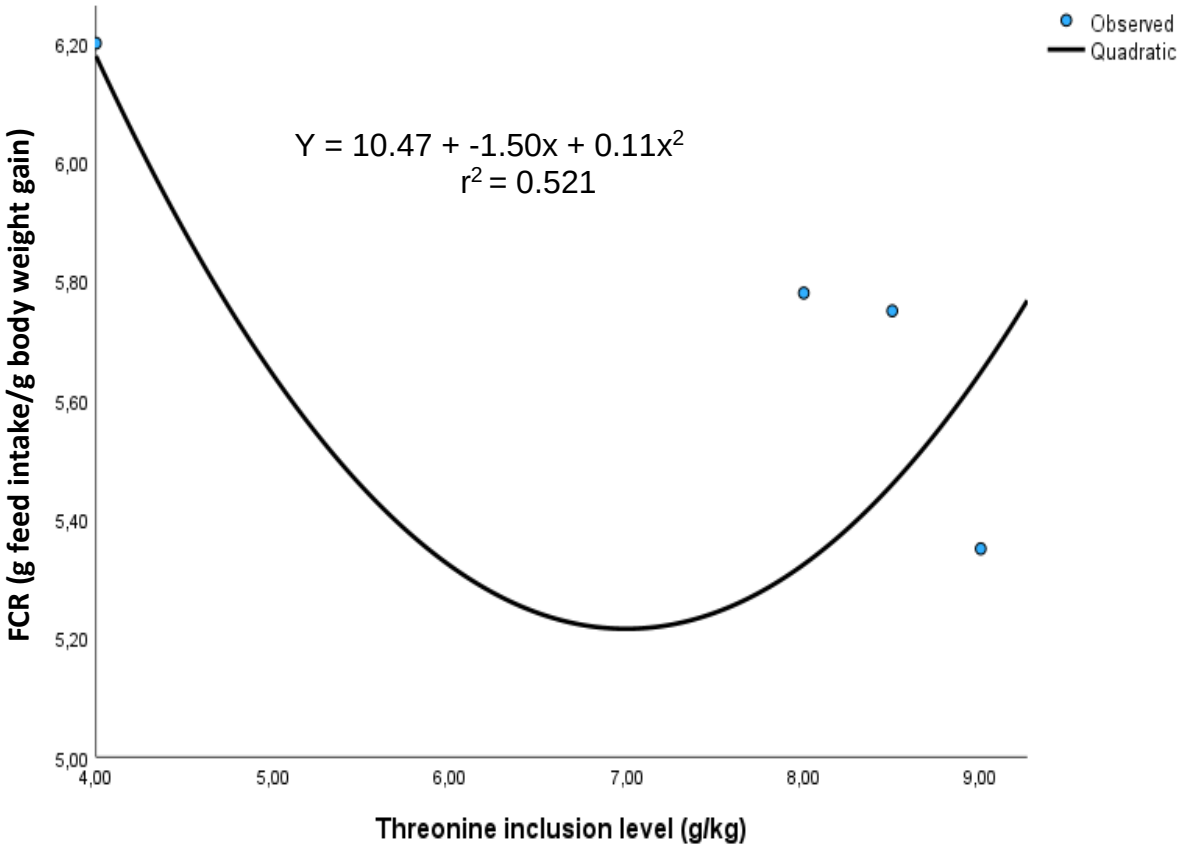

Figure 4.17 The relationship between threonine inclusion level and feed conersion ratio (g feed intake/bird) of male indigenous Boschveld chickens aged 50-91 days

Results on the effect of threonine inclusion levels on gut organ measurements of male indigenous Boschveld chickens aged 91 days are presented in Table 4.10. Threonine inclusion levels had no effect ($p>0.05$) on crop, gizzard, ileum, jejunum, duodenum, caeca, and large intestine digesta pH values of indigenous Boschveld chickens. Similarly, threonine inclusion levels had no effect ($p>0.05$) on GIT, ileum, duodenum, jejunum, and caecum lengths of male indigenous Boschveld chickens aged 91 days. However, threonine inclusion levels had effect ($p<0.05$) on large intestine lengths of indigenous Boschveld chickens. Male indigenous Boschveld chickens on diets containing 7.5 and 8 g of threonine/kg DM had longer ($p<0.05$) large intestine than those on diets having compared to those fed diets containing 8.5 and 9 g threonine per kg DM. However, male chickens fed diets having 7.5 and 8 g of threonine per kg DM had similar ($p>0.05$) large intestine lengths.

Table 4.10 Effect of threonine inclusion levels on gut organ pH, lengths (cm) and weights (g) of male indigenous Boschveld chickens aged 91 days

Variables	Treatment					P-Value
	ATM_4	$ATM_{7.5}$	ATM_8	$ATM_{8.5}$	ATM_9	
Gut organ digesta pH						
Crop	7.00 ±0.106	7.23±0.125	7.08±0.037	7.08±0.081	6.94±0.166	0.1497
Gizzard	7.22±0,018	7.11±0.080	7.16±0.088	7.19±0.018	7.24±0.051	0.4034
Ileum	7.09±0.101	6.97±0.222	7.04±0.152	6.99±0.202	7.07±0.122	0.7359
Jejunum	6.81±0.010	6.86±0.059	6.85±0.160	6.82±0.099	6.84±0.058	0.9824
Ddm	7.03±0.098	6.94±0.206	6.98±0.230	6.96±0.296	7.13±0.049	0.1942
Caeca	7.26±0.191	7.22±0.245	7.39±0.173	7.22±0.023	7.28±0.110	0.9400
LI	7.17±0.102	7.37±0.119	7.11±0.162	7.07±0.201	7.09±0.182	0.0794
Gut organ lengths (cm)						
GIT	143.83±2.720	147.30±6.724	138.97±7.277	143.63±2.617	150.27±4.024	0.5336
Ileum	53.40±1.297	53.40±1.297	53.47±1.934	52.30±2.397	55.10±0.404	0.6134
Jejunum	53.33±1.982	53.67±5.980	51.80±5.184	52.57±5.636	56.07±1.049	0.3328
Ddm	22.27±0.795	23.50±0.436	21.57±1.494	23.20±0.231	23.17±0.884	0.3862
Caeca	32.30±1.471	33.20±1.650	32.87±1.934	33.93±0.698	35.07±1.300	0.6331
LI	6.17^{ab}±0.318	6.87^{a}±0.033	6.30^{a}±0.173	5.00^{c}±0.058	5.40^{bc}±0.173	0.0002
Gut organ weights (g)						
Crop	14.10^{b}±0.866	16.60^{a}±1.443	12.40^{c}±1.375	11.50^{d}±0.764	10.17^{e}±0.088	0.0046
Gizzard	54.17±2.107	48.77±4.140	49.00±3.910	54.67±1.761	46.37±6.540	0.0750
Ileum	16.67±0.644	18.03±0.717	16.83±0.921	15.67±1.644	16.10±1.229	0.4090
Jejunum	22.40^{ab}±1.212	24.07^{a}±1.415	20.77^{ab}±0.426	19.50^{b}±0.231	21.87^{ab}±0.841	0.0551
Ddm	11.90±0.293	11.43±0.774	11.33±0.864	11.37±0.824	12.87±0.677	0.1797
Caeca	8.53±0.565	9.40±0.306	8.93±0.968	7.90±1.195	9.20±0.289	0.3979
LI	2.17±168	2.37±0.033	1.50±0.838	1.77±0.165	2.10±0.238	0.2572

a, b, c, : Means in the same row not sharing a common superscript are significantly different (P<0.05)

ATM: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

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

P-value: Probability value

Threonine inclusion levels had no effect (p>0.05) on gizzard, ileum, duodenum, caecum, and large intestine weights of male indigenous Boschveld chickens aged 91 days. However, threonine inclusion levels had effect (p<0.05) on crop and jejunum

weights of indigenous Boschveld chickens. Male indigenous Boschveld chickens on diets containing 7.5 g of threonine/kg DM had heavier ($p<0.05$) crop weights than other dietary treatments. Male chickens fed diets containing 9 g threonine per kg DM had the lowest ($p<0.05$) crop weight.

Male indigenous Boschveld chickens on a diet containing 7.5 g of threonine per kg DM had heavier ($p<0.05$) jejunum compared to those on diets containing 8.5 g threonine. However, male chickens fed diets having 4, 7.5, 8 and 9 g of threonine per kg of DM had similar ($p>0.05$) jejunum weights. Furthermore, male chickens fed diets having 4, 8, 7.5 and 9 g of threonine/kg DM had similar($p>0.05$) jejunum weights.

Thie large intestine lengths, crop, and jejunum weights were optimized (r^2=0.700, 0.831 and 0.238, respectively) at dietary threonine inclusion levels of 6.08, 5.95, and 5.66, respectively (Figures 4.18 to 4.20, respectively and Table 4.11).

Table 4.11 Threonine meal inclusion levels for optimal gut organ lengths and weights of indigenous Boschveld chickens aged 91 days

Factor	**Formula**	**X**	**Y**	**r^2**	**P**
LI (cm)	$Y = -1.61 + 2.92x + -0.24x^2$	6.08	7.27	0.700	0.300
Crop (g)	$Y = -12.40 + 10.00x + -0.84x^2$	5.95	17.36	0.831	0.169
Jejunum (g)	$Y = 14.88 + 2.92x + -0.26x^2$	5.66	23.08	0.238	0.762

X: Inclusion level for optimal value

Y: Optimal Y-level

r^2: Coefficient of determination

P: Probability

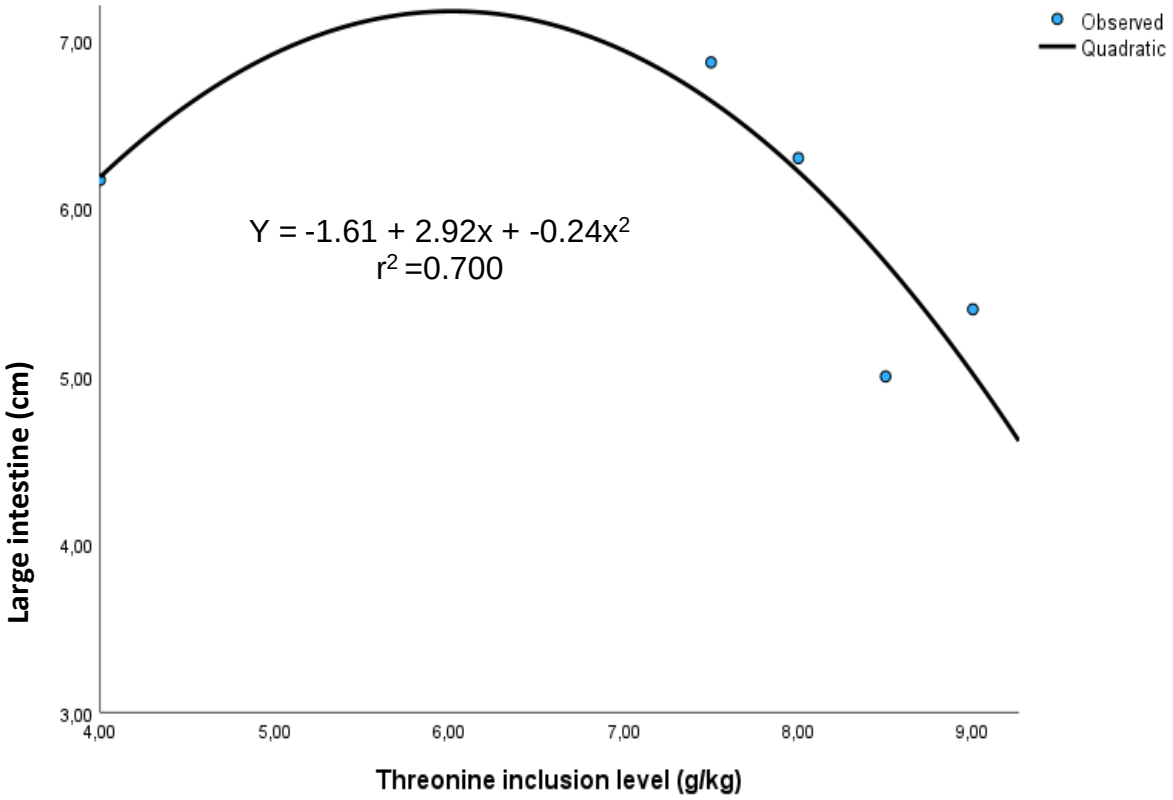

Figure 4.18 Effect of threonine inclusion level on large intestine lengths of male indigenous Boschveld chickens aged 91 days

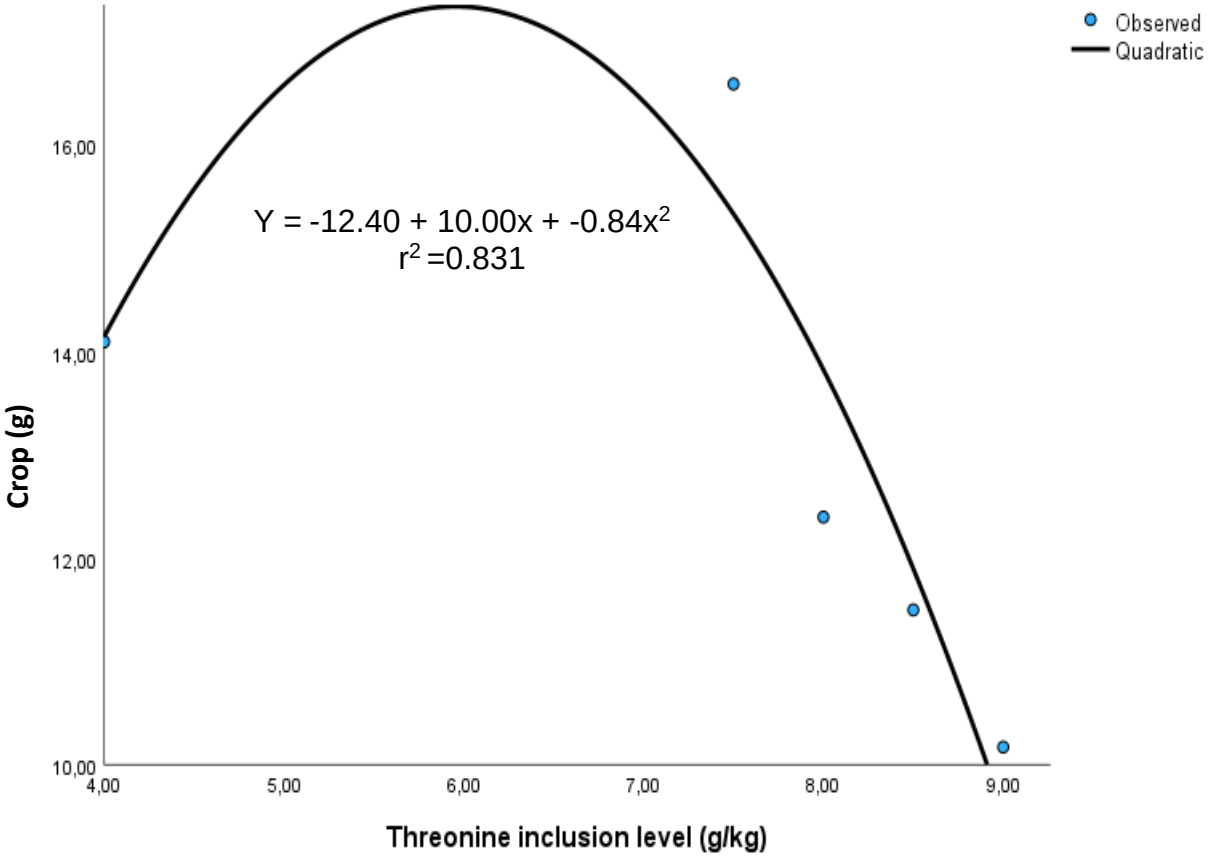

Figure 4.19 Effect of threonine inclusion level on crop weights of male indigenous Boschveld chickens aged 91 days.

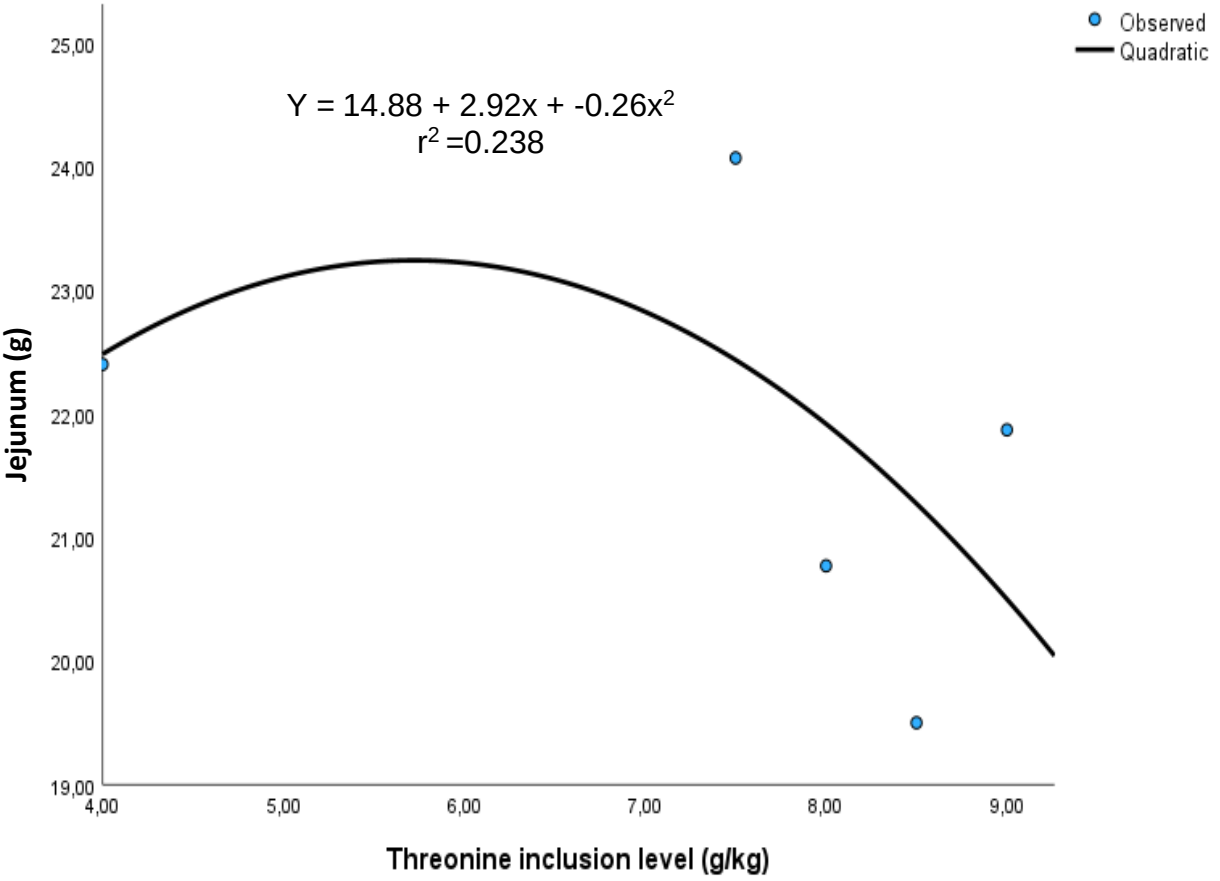

Figure 4.20 Effect of threonine inclusion level on jejunum weights of male indigenous Boschveld chickens aged 91 days

Results on the effect of threonine inclusion levels on meat part weights of male indigenous Boschveld chickens aged 91 days are presented in Table 4.12. Threonine inclusion levels had effect ($p<0.05$) on carcass, thigh, drumstick, breast, and abdominal fat weight of male indigenous Boschveld chickens aged 91 days. Male indigenous Boschveld chickens on diets having 9 and 8.5 g threonine per kg DM had heavier ($p<0.05$) carcass weights than those on diets having 4 and 7.5 g threonine per kg DM. Similarly, male chickens on diets having 8 and 7.5 g threonine per kg DM had heavier ($p<0.05$) carcass weights than those on diets having 4 g of threonine per kg DM. However, chickens fed diets having 9, 8.5 and 8 g of threonine/kg DM similar ($p>0.05$) carcass weights. Similarly, male chickens on diets having 7.5 and 8 g of threonine per kg DM had the same ($p>0.05$) carcass weights.

Male indigenous Boschveld chickens on diets containing 8.5 g of threonine per kg DM had heavier ($p<0.05$) thighs than other dietary treatments while those on 4 g threonine

had the lowest ($p<0.05$) thigh weight. However, male chickens fed diets having 7.5, 8 and 9 g of threonine/kg DM had similar ($p>0.05$) thigh weights.

Male indigenous Boschveld chickens on a diet having 9 g of threonine/kg DM had heavier ($p<0.05$) drumstick weights than their counterparts on diets containing 4, 7.5, 8 and 8.5 g of threonine/kg DM. Male chickens fed diets containing 8, 8.5 and 7.5 threoine kg per DM had heavier ($p<0.05$) drumstick weights compared those on a diet having 4 g of threonine/kg DM. However, chickens fed on diets having 7.5, 8 and 8.5 g of threonine per kg DM had similar ($p>0.05$) drumstick weights.

Male indigenous Boschveld chickens on a diet having 9 g of threonine/kg DM had heavier ($p<0.05$) breast weights than other dietary treatments. On the other hand, chickens on diets containing 8 g threonine per kg DM had the lowest ($p<0.05$) breast weight compared to their counterparts. Furthermore, male chickens on a diet containing 8.5 g of threonine/kg DM had heavier ($p<0.05$) breast weights than those on diets having 4, 7.5 and 8 g of threonine/kg DM. Male chickens on a diet containing 4 g of threonine/kg DM had heavier ($p<0.05$) breast weights than those on diets having 7.5 and 8 g of threonine/kg DM. Similarly, male chickens on a diet containing 7.5 g of threonine/kg DM had heavier ($p<0.05$) breast weights than those on a diet having 8 g of threonine/kg DM.

Male indigenous Boschveld chickens on a diet having 7.5 g of threonine/kg DM had heavier ($p<0.05$) abdominal fat weights than those on diets containing 4, 8 and 8.5 g of threonine/kg DM. However, male chickens fed on diets having 7.5 and 9 g of threonine per kg DM had similar ($p>0.05$) abdominal fat weights. Similarly, male chickens on diets containing 8 and 9 g of threonine/kg DM had heavier ($p<0.05$) abdominal fat weights than those on diets having 4 and 8.5 g of threonine/kg DM. Similarly, male chickens fed on diets having 8 or 9 g of threonine per kg DM had the same ($p>0.05$) abdominal fat weight.Again, birds fed diets containing 4 and 8.5 g threonine per kg DM had similar abdominal fat weight.

Table 4.12 Effect of threonine inclusion levels on meat part weights (g) of male indigenous Boschveld chickens aged 91 days

Variable	Treatment					P-Value
	ATM_4	$ATM_{7.5}$	ATM_8	$ATM_{8.5}$	ATM_9	
Carcass	936.67^{c}±15.502	1056.77^{b}±23.527	1117.60^{ab}±60.456	1138.77^{a}±35.940	1188.50^{a}±21.939	0.0046
Thigh	62.90^{c}±0.058	75.70^{b}±1.552	75.67^{b}±2.396	82.67^{a}±0.088	77.30^{b}±0.577	<.0001
Drumstick	70.30^{c}±1.155	76.53^{b}±1.795	75.07^{b}±3.320	77.30^{b}±0.866	82.03^{a}±0.371	0.0138
Breast	166.57^{c}±0.145	164.20^{d}±2.223	148.07^{e}±4.474	172.17^{b}±1.241	191.57^{a}±1.588	<.0001
Abdominal fat	12.17^{c}±0.376	19.00^{a}±1.443	16.00^{b}±0.577	12.00^{c}±0.751	16.67^{ab}±1.819	0.0055

a, b, c, d e: Means in the same row not sharing a common superscript are significantly different (P<0.05)

ATM: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

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

Positive relationships were observed between threonine inclusion level and carcass, thigh, drumstick, and breast weights (r^2 = 0.947, 0.870, 0.801, and 0.078, respectively) of male indigenous Boschveld chickens aged 91 days (Figures 4.21 to 4.24, respectively and Table 4.13). Abdominal fats of male indigenous Boschveld chickens aged 91 days were optimized (r^2= 0.396) at a threonine inclusion level of 6.87 g/kg DM of the diet (Figure 4.25).

Table 4.13 Relationships between threonine inclusion level and meat parts weight of male indigenous Boschveld chickens aged 91 days

Factor	Formula	r^2	Probability
Carcass (g)	Y = 47.50x + 736.14	0.947	0.005
Thigh (g)	Y = 3.42x + 49.52	0.870	0.021
Drumstick (g)	Y = 1.91x + 62.13	0.801	0.040
Breast (g)	Y = 2.22x + 152.10	0.078	0.648

r^2: Coefficient of determination

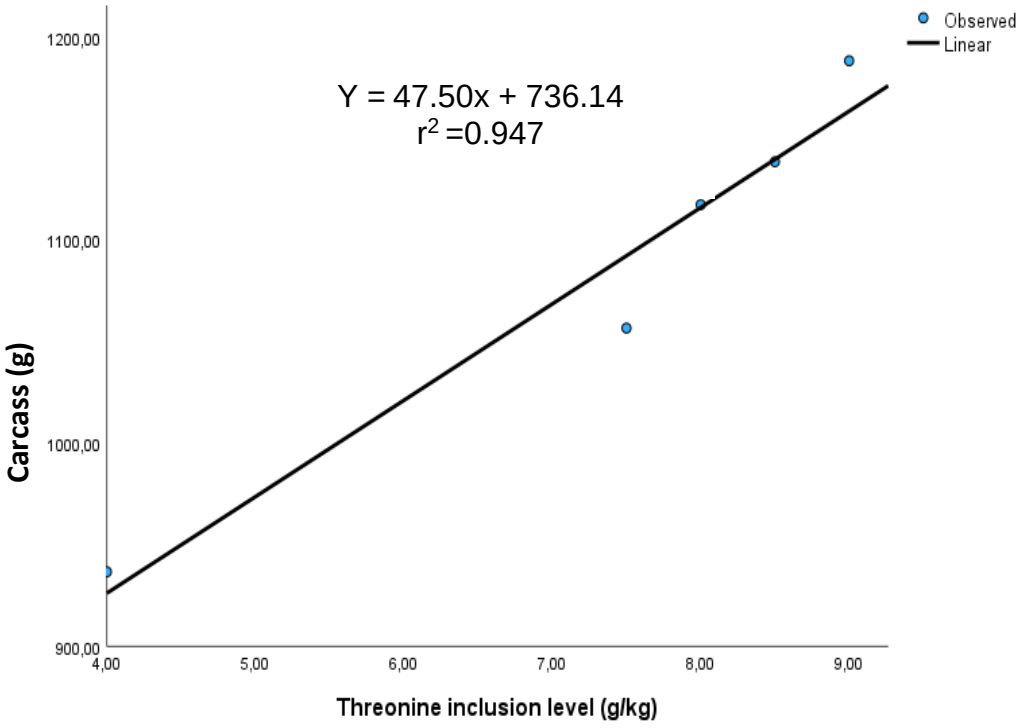

Figure 4.21 The relationship between threonine inclusion level and carcass weights of male indigenous Boschveld chickens aged 91 days

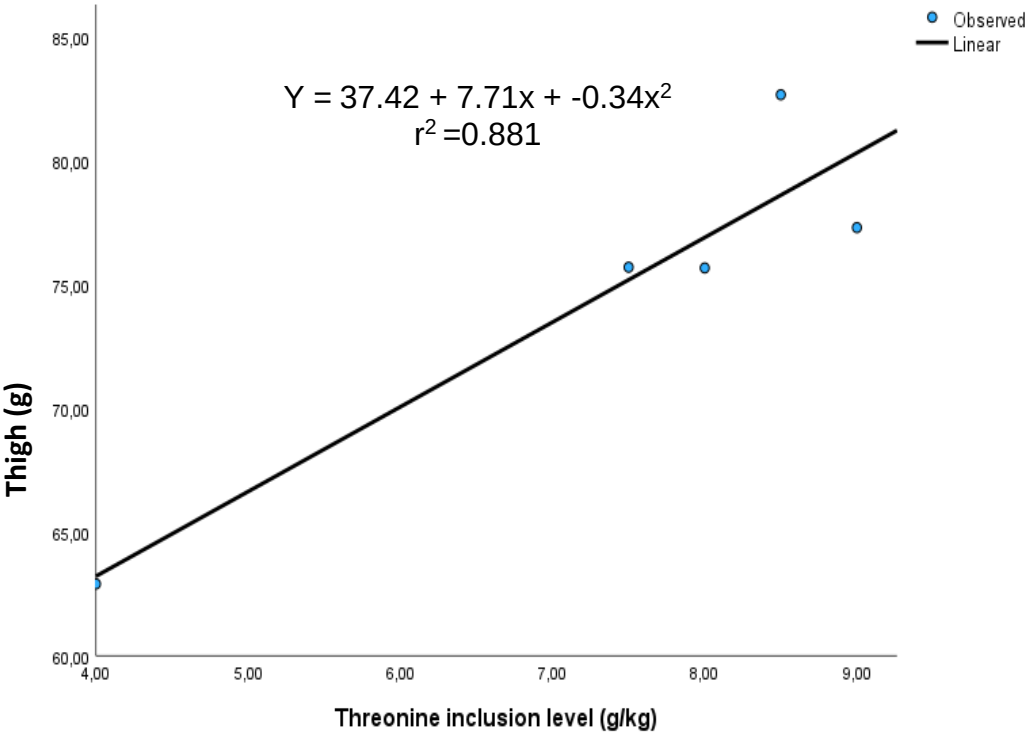

Figure 4.22 The relationship between threonine inclusion level and thigh weights of male indigenous Boschveld chickens aged 91 days

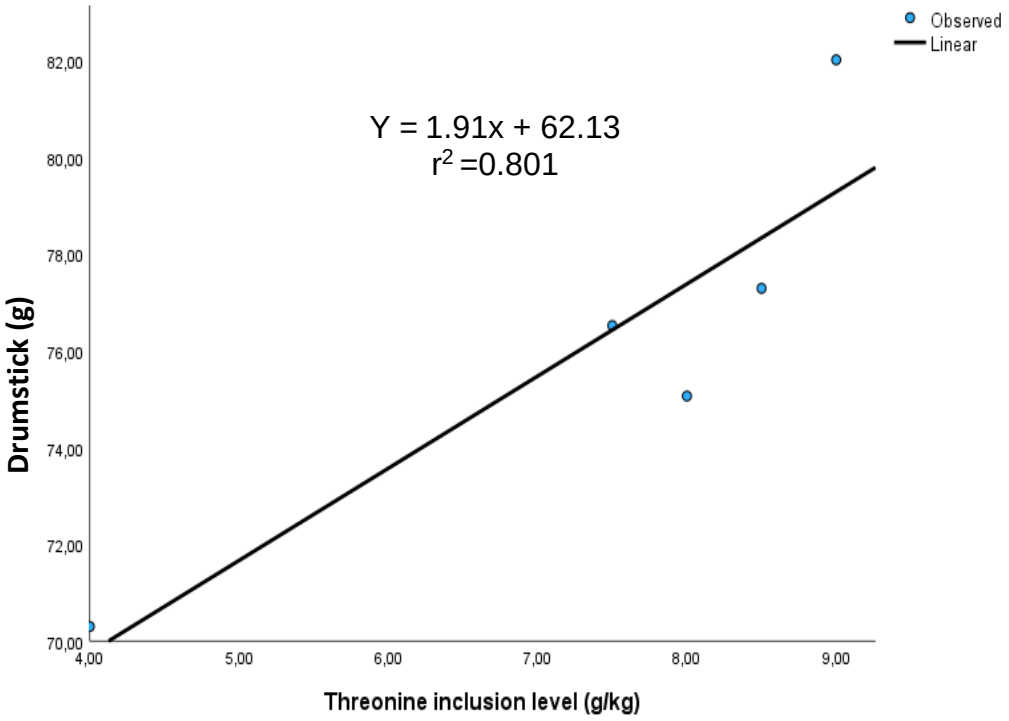

Figure 4.23 The relationship between threonine inclusion level and drumstick weights of male indigenous Boschveld chickens aged 91 days

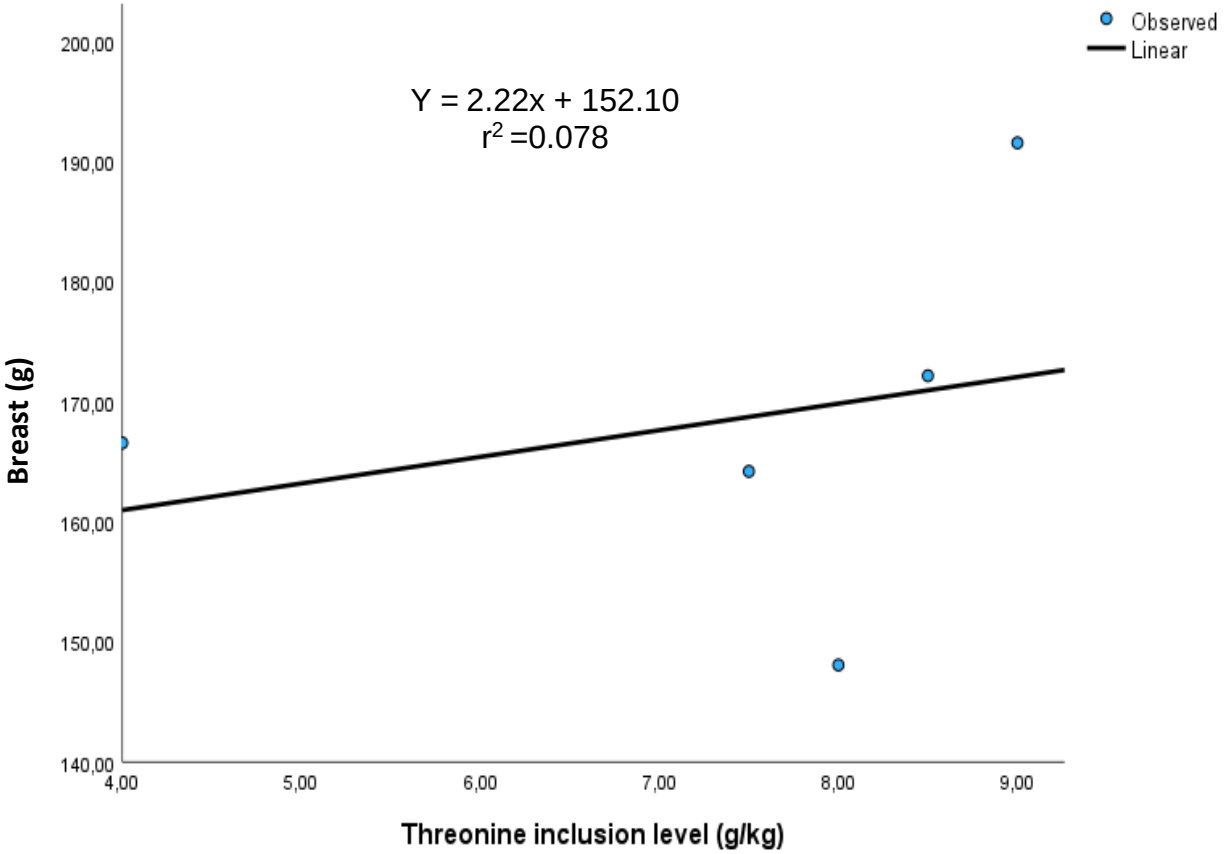

Figure 4.24 The relationship between threonine inclusion level and breast weights of male indigenous Boschveld chickens aged 91 days

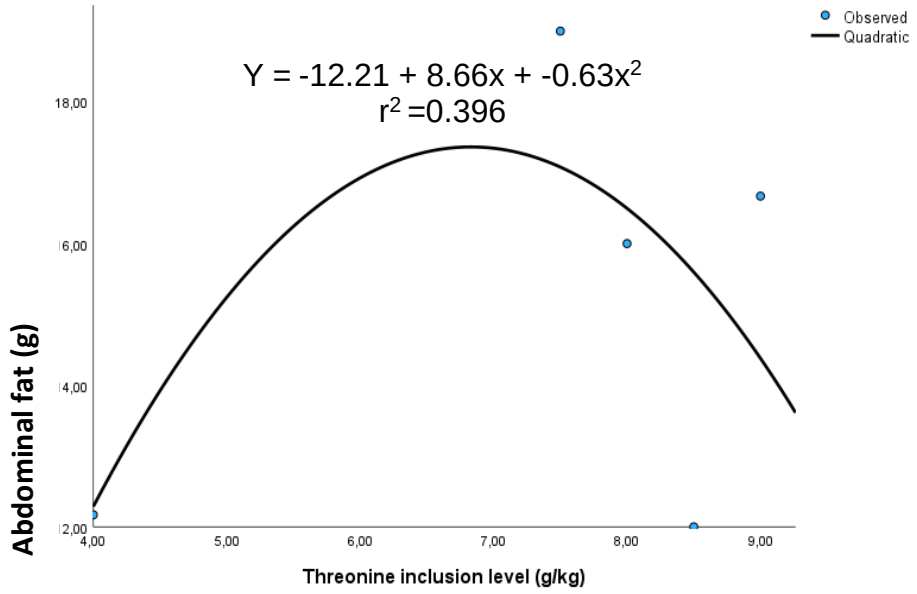

Figure 4.25 Effect of threonine inclusion level on abdominal fat weights of male indigenous Boschveld chickens aged 91 days

Results on the effect of threonine inclusion levels on breast colour and pH values of male indigenous Boschveld chickens aged 91 days are presented in Table 4.14. Threonine inclusion levels had effect ($p<0.05$) on breast L*(lightness), a*(redness), b*(yellowness), C*(chroma) and h (hue angle) of male indigenous Boschveld chickens aged 91 days. However, threonine inclusion levels had no effect ($p>0.05$) on breast pH.

Male indigenous Boschveld chickens on diets having 8 and 8.5 g of threonine per kg DM had lighter ($p<0.05$) breasts than those on diets having 9, 7.5 and 4 g of threonine/kg DM. However, chickens fed diets containing 8 and 8.5 g threonine had similar ($p>0.05$) breast colour. Male chickens on a diet having 9 g of threonine per kg DM had lighter ($p<0.05$) breasts than those on diets having 7.5 and 4 g of threonine/kg DM. Similarly, male chickens on a diet having 7.5 g of threonine per kg DM had lighter ($p<0.05$) breasts than those on a diet containing 4 g of threonine/kg DM.

Male indigenous Boschveld chickens on a diet having 4 g of threonine/kg DM had higher ($p<0.05$) breast redness values than other dietary treatments. Male chickens on a diet having 9 g of threonine/kg DM had higher ($p<0.05$) breast redness values than those on diets containing 7.5, 8 and 8.5 g of threonine/kg DM. Male indigenous Boschveld chickens on a diet containing 7.5 g of threonine/kg DM had higher ($p<0.05$) breast redness values than those on diets having 8 and 8.5 g of threonine/kg DM. Similarly, male chickens on a diet containing 8 g of threonine/kg DM had higher ($p<0.05$) breast redness values than those on a diet having 8.5 g of threonine/kg DM. Chickens fed diets containing 8 g threonine per kg DM had lower ($p<0.05$) redness values than other treatments).

Male indigenous Boschveld chickens on a diet containing 4 g of threonine/kg DM had higher ($p<0.05$) breast yellowness values than other treatments. Male chickens on a diet having 7.5 g of threonine/kg DM had higher ($p<0.05$) breast yellowness values than those on diets containing 8, 8.5 and 9 g of threonine/kg DM. Male indigenous Boschveld chickens on a diet containing 8 g of threonine/kg DM had higher ($p<0.05$) breast yellowness values than those on diets having 8.5 and 9 g of threonine/kg DM. Similarly, male chickens on a diet containing 9 g of threonine/kg DM had higher ($p<0.05$) breast yellowness values than those on a diet having 8.5 g of threonine/kg

DM. In this study, chickens fed diets containing 8.5 g threonine/kg DM had lower ($p<0.05$) yellowness values than other treatments.

Table 4.14 Effect of threonine inclusion levels on breast colour and pH of male indigenous Boschveld chickens aged 91 days

Variable	Treatment					P-Value
	ATM_4	$ATM_{7.5}$	ATM_8	$ATM_{8.5}$	ATM_9	
Lightness	52.24^{d}±0.557	56.39^{c}±0.159	65.91^{a}±0.465	65.96^{a}±0.537	61.68^{b}±0.958	<.0001
Redness	6.83^{a}±0.156	4.81^{c}±0.081	3.98^{d}±0.087	3.80^{e}±0.023	6.24^{b}±0.062	<.0001
Yellowness	12.21^{a}±0.167	11.43^{b}±0.240	8.50^{c}±0.256	5.53^{e}±0.095	7.95^{d}±0.225	<.0001
Chroma	14.04^{b}±0.228	12.51^{c}±0.167	9.98^{e}±0.274	14.82^{a}±0.032	11.50^{d}±0.427	<.0001
Hue angle	60.87^{d}±0.566	64.78^{b}±0.502	58.49^{e}±0.286	72.58^{a}±1.316	62.57^{c}±0.828	<.0001
Breast pH	6.87±0.067	6.80±0.058	6.87±0.067	6.77±0.033	6.80±0.058	0.6712

a, b, c, d, e : Means in the same row not sharing a common superscript are significantly different (P<0.05)

ATM: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine inclusion levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

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

Male indigenous Boschveld chickens on a diet containing 8.5 g of threonine/kg DM had higher ($p<0.05$) breast chroma values than those on diets having 4, 7.5, 8 and 9 g of threonine/kg DM. Male chickens on a diet having 4 g of threonine/kg DM had higher ($p<0.05$) breast chroma values than those on diets containing 7.5, 8 and 9 g of threonine/kg DM. Male indigenous Boschveld chickens on a diet containing 7.5 g of threonine/kg DM had higher ($p<0.05$) breast chroma values than those on diets having 8 and 9 g of threonine/kg DM. Similarly, male chickens on a diet containing 9 g of threonine/kg DM had higher ($p<0.05$) breast chroma values than those on a diet having 8 g of threonine/kg DM. Chickens fed 8 g threonine per kg DM had lower ($p<0.05$) chroma values compared to other dietary treatments.

As is the case with chroma, male Boschveld chickens on a diet containing 8.5 g of threonine/kg DM had higher ($p<0.05$) breast hue angle values than those on diets having 4, 7.5, 8 and 9 g of threonine/kg DM. Male chickens on a diet having 7.5 g of threonine/kg DM had higher ($p<0.05$) breast hue angle values than those on diets containing 4, 8 and 9 g of threonine/kg DM. Male indigenous Boschveld chickens on a diet containing 9 g of threonine/kg DM had higher ($p<0.05$) breast hue angle values than those on diets having 4 and 8 g of threonine/kg DM. Similarly, male chickens on a diet containing 4 g of threonine/kg DM had higher ($p<0.05$) breast hue angle values than those on a diet having 8 g of threonine/kg DM. Breast meat from birds fed 8 g threonine/kg DM had lower hue values compared to other treatments.

A positive relationship was observed between threonine inclusion level and breast lightness and hue angle (r^2 = 0.659 and 0.116, respectively) of male indigenous Boschveld chickens aged 91 days (Figure 4.26, 4.30, respectively and Table 4.15). However, negative relationships were observed between threonine inclusion level and breast redness, yellowness and chroma (r^2 = 0.585, 0.333 and 0.142 and, respectively) of male indigenous Boschveld chickens aged 91 days (Table 4.15 and Figures 4.27, 4.28 and 4.29, respectively).

Table 4.15 Relationships between threonine inclusion level and breast colour of male indigenous Boschveld chickens aged 91 days

Factor	Formula	r^2	Probability
L* (lightness)	Y = 2.47x + 42.14	0.659	0.095
b* (yellowness)	Y = -1.05 + 16.89	0.585	0.132
a* (redness)	Y = -0.39x + 8.05	0.333	0.308
C* (chroma)	Y = -0.37x + 15.30	0.142	0.532
h (hue angle)	Y = -0.93x + 56.99	0.116	0.575

r^2: Coefficient of determination

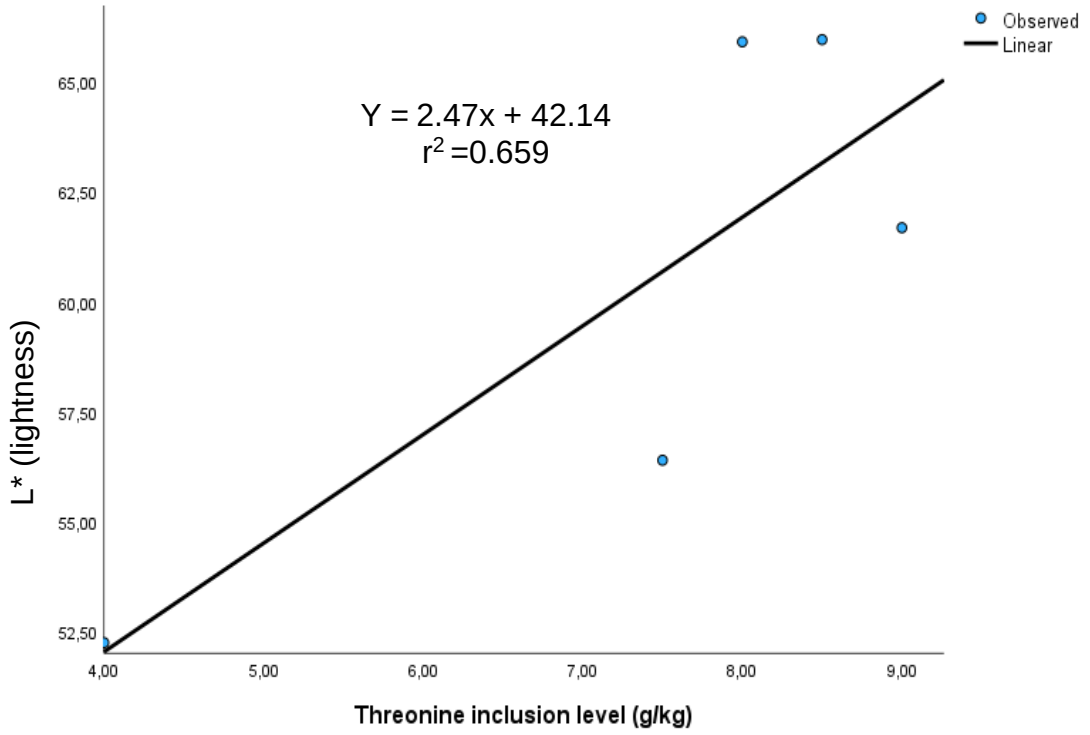

Figure 4.26 The relationship between threonine inclusion level and breast lightness of male indigenous Boschveld chickens aged 91 days

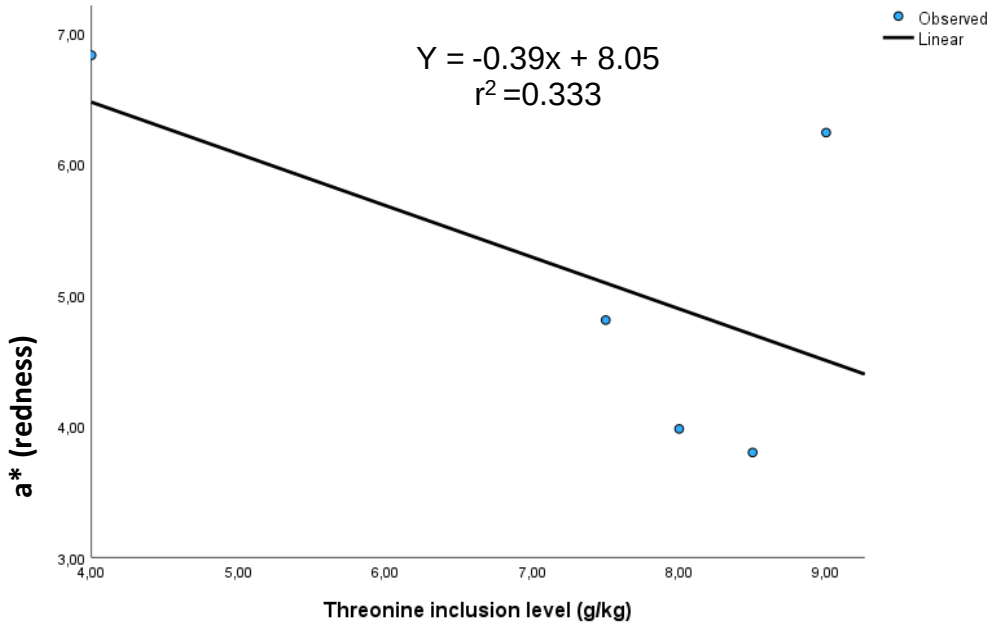

Figure 4.27 The relationship between threonine inclusion level and breast redness of male indigenous Boschveld chickens aged 91 days

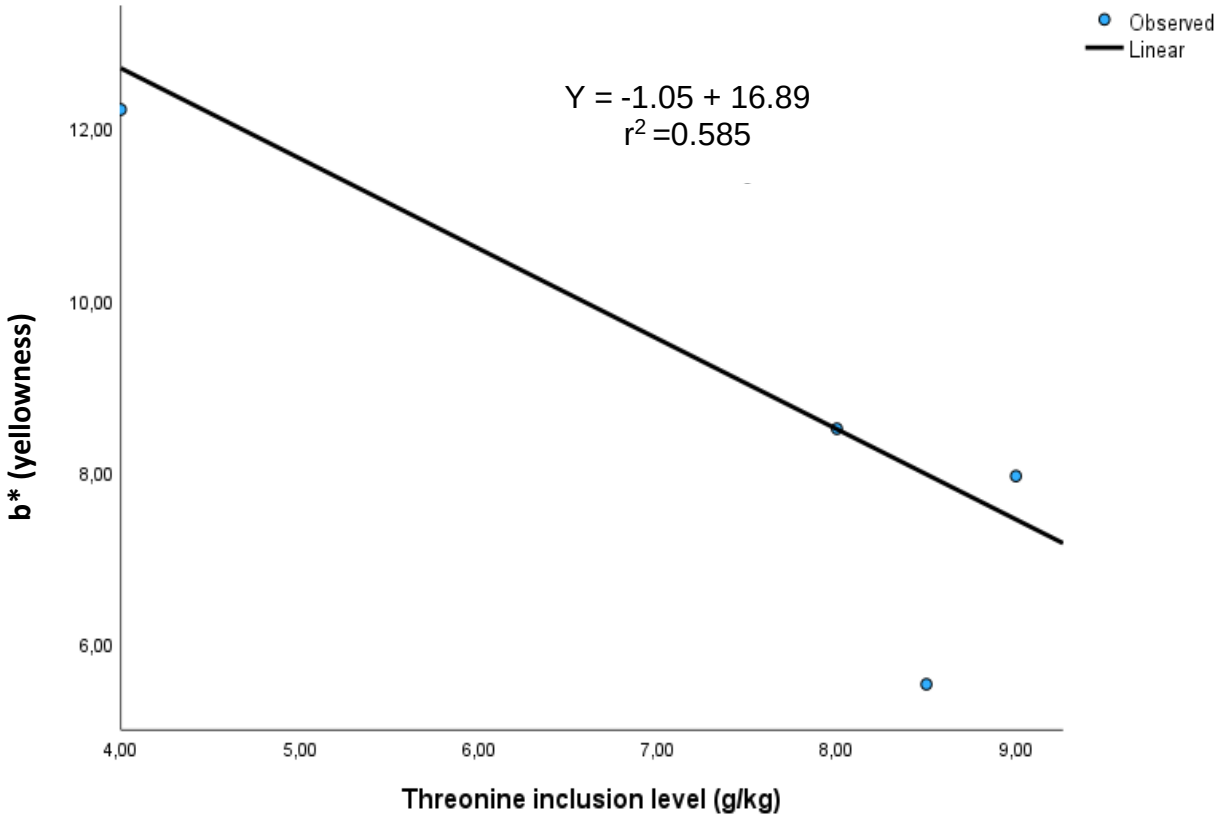

Figure 4.28 The relationship between threonine inclusion level and breast yellowness of male indigenous Boschveld chickens aged 91 days

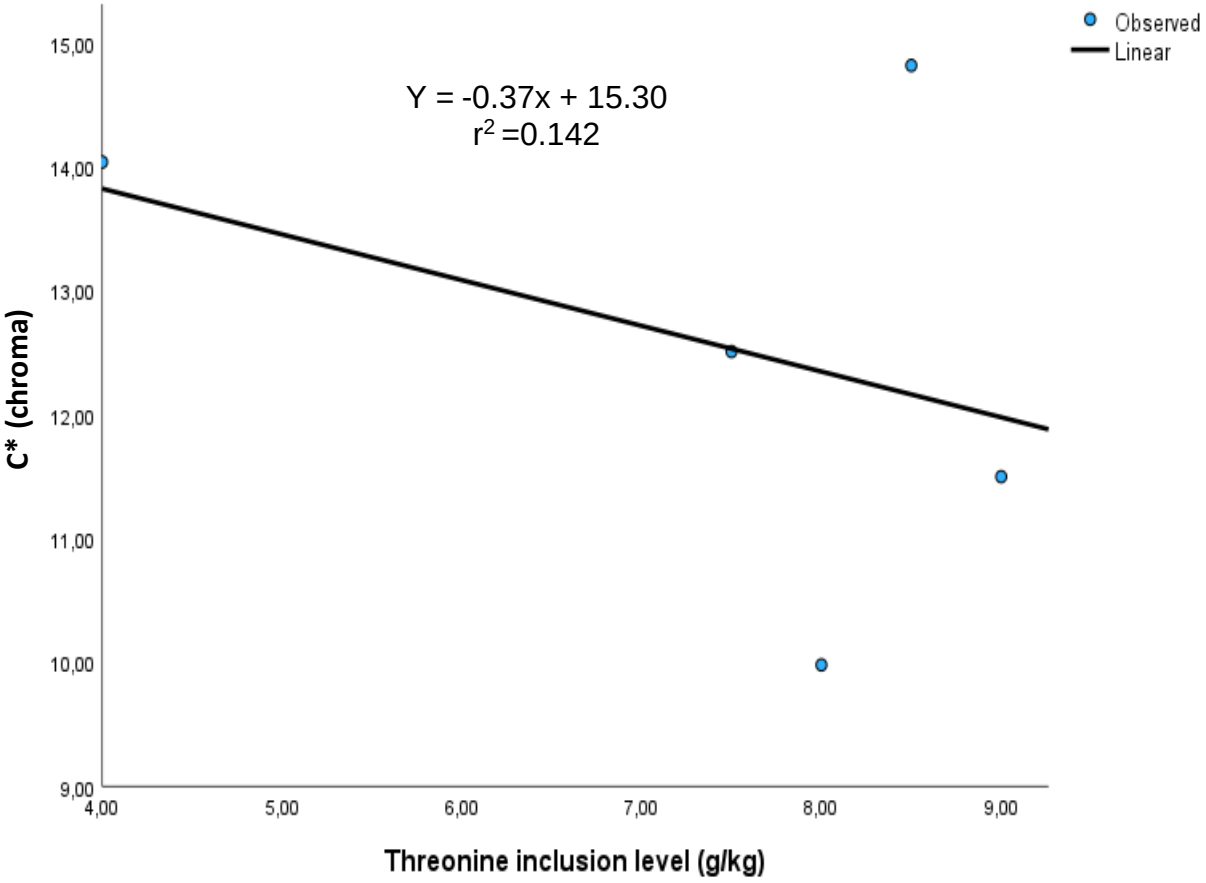

Figure 4.29 The relationship between threonine inclusion level and breast chroma of male indigenous Boschveld chickens aged 91 days

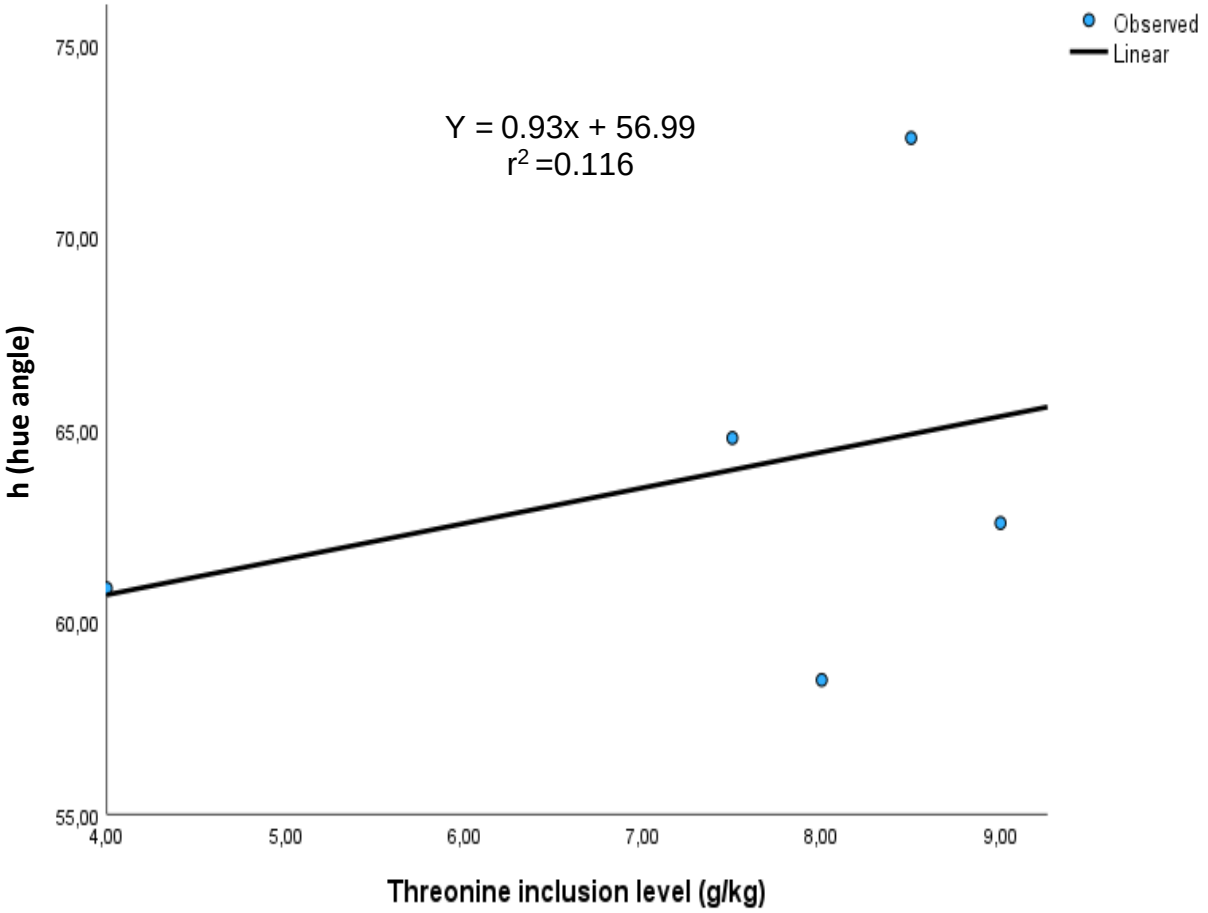

Figure 4.30 The relationship between threonine inclusion level and breast hue angle of male indigenous Boschveld chickens aged 91 days

Results on the effect of threonine inclusion levels on meat sensory attributes, and cooking loss of male indigenous Boschveld chickens aged 91 days are presented in Table 4.16. Threonine inclusion levels had effect ($p<0.05$) on breast meat tenderness. However, threonine inclusion levels in a diet had no effect ($p>0.05$) on Boschveld chicken meat juiciness, flavour, overall acceptability, and cooking loss values. Male indigenous Boschveld chickens on diets having 8.5 and 9 g of threonine/kg DM had higher ($p<0.05$) meat tenderness values than those on diets having 8, 7.5 and 4 g of threonine per kg DM. Chickens on diets containing 8 and 7.5 g threonine/kg DM had higher ($p<0.05$) meat tenderness values than those on diets having 4 g of threonine per kg DM. However, male chickens on diets having 8.5 and 9 g of threonine/kg DM had similar ($p>0.05$) meat tenderness values. Similarly, male chickens on diets having 7.5 and 8 g of threonine/kg DM had the same ($p>0.05$) meat tenderness values. Birds fed 4 g threonine had the lowest ($p<0.05$) tenderness values than other dietary treatments.

Table 4.16 Effect of threonine inclusion levels on meat sensory attributes, and cooking loss of male indigenous Boschveld chickens aged 91 days

Variable	**Treatment**					P-Value
	ATM_4	**$ATM_{7.5}$**	**ATM_8**	**$ATM_{8.5}$**	ATM_9	
Tenderness	2.33^{c}±0.333	3.00^{b}±0.000	3.00^{b}±0.000	3.67^{a}±0.333	4.00^{a}±0.000	0.0019
Juiciness	3.67±0.333	3.33±0.333	3.33±0.333	3.00±0.664	4.00±0.338	0.1466
Flavour	3.67±0.333	3.33±0.333	3.33±o.333	3.67±0.333	3.67±0.333	0.8714
Overall acceptability	3.33±0.333	3.33±0.333	3.00±0.338	3.67±0.333	3.67±0.333	0.5121
Cooking loss	35.82±2.518	32.45±5.921	30.54±8.600	24.89±10.413	32.00±3.753	0.6490

a, b, c, d : Means in the same row not sharing a common superscript are significantly different (P<0.05)

ATM: Treatment codes are explained in Chapter 3, Table 3.01 (The treatments were dietary threonine inclusion levels of 4, 7.5, 8.0, 8.5 or 9.0 g/kg DM of feed)

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

A positive relationship was observed between threonine inclusion level and meat tenderness values (r^2 = 0.799) of male indigenous Boschveld chickens aged 91 days (Figure 4.31).

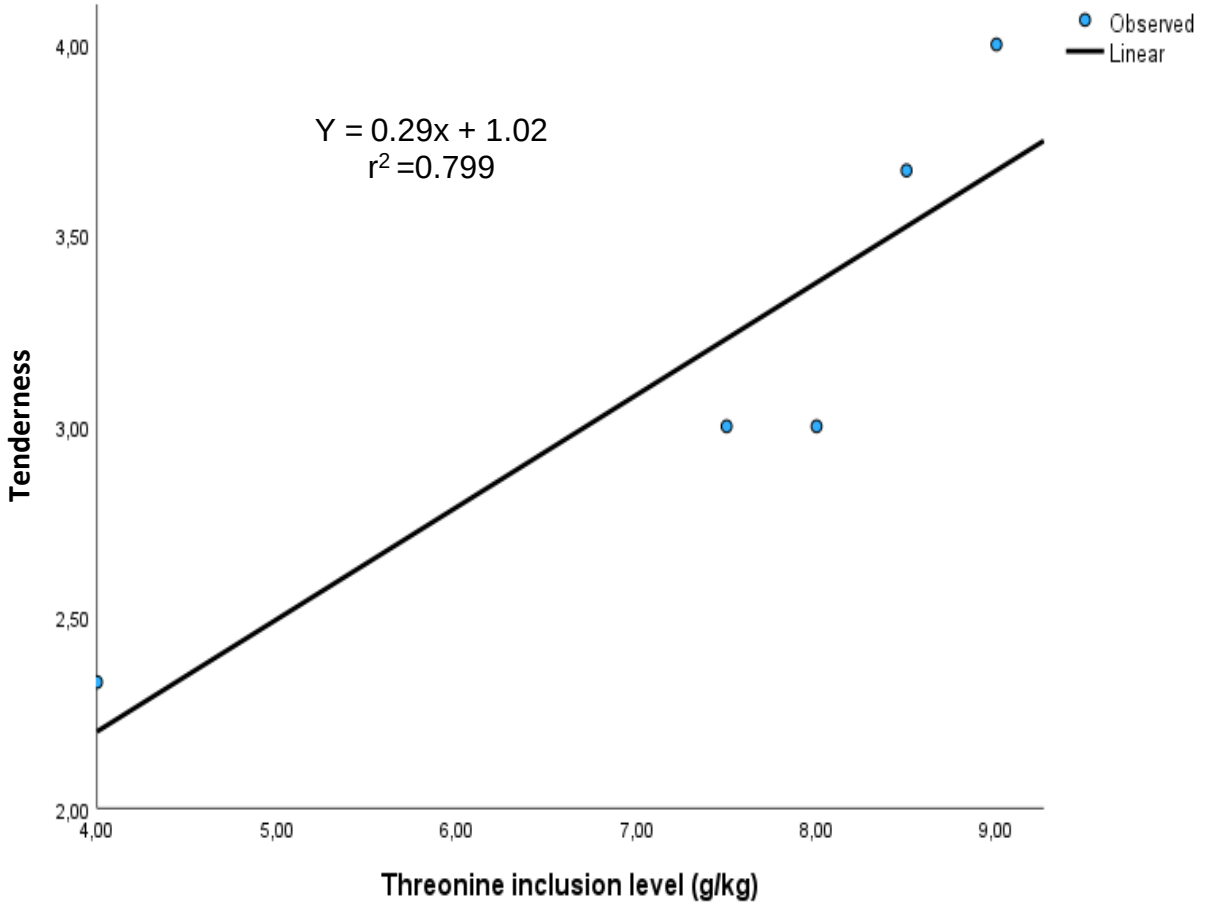

Figure 4.31 The relationship between threonine inclusion level and breast tenderness of male indigenous Boschveld chickens aged 91 days

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

5.1.1 Experiment 1 (Indigenous Boschveld chickens aged one to 49 days).

Results of the nutrient composition of the experimental diets are presented in Table 4.01 in Chapter 4 of this thesis. 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 at starter phase. Thus, any difference in response should be due to dietary threonine level.

Results of the current study indicated that dietary threonine levels had effect on feed intake (g DM/bird/day), BWG (g/bird/day), and FCR (g feed intake/g BWG) of unsexed indigenous Boschveld chickens aged 1-49 days. Unsexed indigenous Boschveld chickens aged 1-49 days fed diets having 8 g of threonine/kg DM had higher feed intakes than those on diets having 4, 7.5, 8.5 and 9 g of threonine/kg DM. A negative relationship between threonine and feed intake was observed. However, the results of the current study are in line with those of Ng'ambi *et al.* (2017) who observed significant differences in feed intakes (g DM/bird/day) of Venda chickens aged 1-49 days fed diets having threonine inclusion level at 6.218 g/kg DM. Moreover, Samadi (2006) observed a significant difference in feed intakes (g DM/bird/day) fed dietary threonine level of 7.9 g per kg DM for optimal intake of 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.

Unsexed indigenous Boschveld chickens aged 1-49 days on diets having 9 g of threonine/kg DM had heavier BWGs than those on diets having 4, 7.5, 8 and 8.5 g of threonine/kg DM. A positive relationship between threonine and BWG was observed. The results of the current study are in line with Estalkhzir *et al.* (2013) who observed a significant difference in BWG fed dietary threonine level of 0.0011 g/kg DM of broiler Japanese quail from 1 to 35 days old. NRC (1994) recommended a dietary threonine level of 8.0 g/kg DM for optimal BWG for broiler chickens aged 1 to 21 days.

Dietary threonine level of 12.00 g/kg DM optimized FCR of unsexed indigenous Boschveld chickens. The current results are in line with NRC (1994). NRC reported a significant difference on FCR value in broilers aged 1-21 days and fed dietary threonine level of 7.4 g/kg DM. Ng'ambi *et al.* (2017) observed significant difference

on the FCR of unsexed Venda chickens aged 8 to 13 weeks fed dietary threonine level of 6.442 g/kg DM.

Results of the current study indicated that dietary threonine levels had no effect on crop, gizzard, ileum, jejunum, caeca, and large intestine pH values of unsexed indigenous Boschveld chickens aged 49 days. However, the present results showed that dietary threonine levels influenced duodenum pH value of unsexed indigenous Boschveld chickens aged 49 days. Dietary threonine level of 4.52 g/kg DM optimized duodenum pH in unsexed indigenous Boschveld chickens aged 49 days. The current results disagree with Ng'ambi *et al.* (2017) who observed a significant difference in proventriculus pH values of unsexed Venda chickens aged 91 days and fed dietary threonine level of 5.7 g/kg DM. The authors observed no significant difference at 6.2 and 5.9 g/kg DM threonine inclusion levels on the small intestines, large intestines, and caecum pH values of unsexed Venda chickens aged 91 days. According to Dibner (2004), low pH in the proventriculus is required for adequate protein digestion and general reduction in bacteria community in the different segments of the gut. Choct (2009) reported that high pH in the gut results in the proliferation of pathogenic microbiota which is detrimental to both the growth and health of chickens. Ao *et al.* (2008) stated that gut organ pH for chickens varies between 5.5 and 6.5 and changes as digesta transit different segments of the GIT.

The present results indicate that threonine inclusion levels had effect on GIT, ileum jejunum, duodenum, and large intestine lengths of unsexed indigenous Boschveld chickens aged 49 days. However, threonine inclusion levels had no effect on caeca lengths of indigenous Boschveld chickens aged 49 days. The difference should be due to dietary threonine levels.

Dietary threonine levels did not improve GIT, ileum, jejunum, duodenum lengths of unsexed indigenous Boschveld chickens. However, threonine improved large intestine lengths of unsexed indigenous chickens aged 49 days. These results are inconsistent with Moghaddam *et al.* (2011) who observed significant differences on optimal gut organ lengths of broiler chickens aged 1 to 14 days and fed dietary threonine level of 8.7 g/kg DM. Ng'ambi *et al.* (2019) observed significant differences in proventriculus, and large intestine weights fed dietary threonine levels of 7.5 and 9.1 g/kg DM of

unsexed Ross 308 broiler chickens aged 42 days. NRC (1994) recommended a dietary threonine level of 8.0 g/kg DM for optimal gut organ lengths for broiler chickens aged 1 to 21 days.

Results of the current study indicate that threonine inclusion levels had effect on crop, gizzard, ileum jejunum, duodenum, and caeca weights of unsexed indigenous Boschveld chickens aged 49 days. However, threonine inclusion had no effect on large intestine weights of unsexed indigenous Boschveld chickens aged 49 days. Dietary threonine level of 8.00 g/kg DM optimized duodenum weight of unsexed indigenous Boschveld chickens. Dietary threonine levels did not improve crop, gizzard, ileum, jejunum, and caeca weights of unsexed indigenous Boschveld chickens. The results of the current study are in line with Ng'ambi *et al.* (2019) who observed a significant difference in proventriculus weight fed dietary threonine level of 7.5 g/kg DM of unsexed broiler chickens aged 42 days. Similarly, Ngomani *et al.* (2019) observed a significant difference in large intestine weights fed dietary threonine levels of 9.1 g/kg DM of unsexed broiler chickens aged 42 days. Moreover, El-Faham *et al*. (2017) observed significant differences in optimal gut organ weights fed threonine level of 8.7 g/kg DM of broiler chickens aged 21 to 42 days.

5.1.2 Experiment 2 (Indigenous Boschveld chickens aged 50 to 91 days).

The current results indicate that threonine inclusion levels had effect on feed intake (g DM/bird/day), BWG (g/bird/day), and FCR (g feed intake/g BWG) of male indigenous Boschveld chickens aged 50-91 days.

Male indigenous Boschveld chickens aged 50-91 days on diets having 4 g/kg threonine inclusion level had higher feed intake than those on diets having 7.5, 8, 8.5 and 9 g of threonine/kg DM. A negative relationship between threonine level and feed intake was observed. The results disagree to the results of Ng'ambi *et al.* (2017) who observed significant differences in feed intake (g DM/bird/day) of Venda chickens aged 1-49 days fed diets having threonine inclusion level at 6.218 g/kg DM. Moreover, Samadi (2006) observed a significant difference in feed intake (g DM/bird/day) of broiler chickens aged 1-21 days and fed dietary threonine level of 7.9 g per kg DM for optimal

intake. Thomas (2008) recommended a threonine level of 6.9 g/kg DM for optimal intake of broiler chickens aged 14 to 28 days.

Dietary threonine level of 6.12 g/kg DM optimized BWG of male indigenous Boschveld chickens aged 50-91 days. However, the present results disagree with those of Estalkhzir *et al.* (2013) who observed a significant difference in BWG of broiler Japanese quail from 1 to 35 days old and fed dietary threonine level of 0.0011 g/kg DM. NRC (1994) recommended a dietary threonine level of 8.0 g/kg DM for optimal BWG for broiler chickens aged one to 21 days.

Dietary threonine level of 6.82 g/kg DM optimized FCR of male indigenous Boschveld chickens aged 50-91 days. However, the results of the current study are incosistent with NRC (1994). NRC found better significant difference on FCR value in broiler chickens aged 1 to 21 days and fed dietary threonine level of 7.4 g/kg DM. Moreover, Valizade *et al.* (2016) and Kheiri and Alibeyghi (2017) observed poor FCR value in broiler chickens aged 1 to 21 days and fed dietary threonine levels of 8.4 and 9.0 g/kg DM.

Results of this study indicate that threonine inclusion levels had no effect on crop, gizzard, ileum, jejunum, duodenum, caeca, and large intestine pH values of indigenous male Boschveld chickens aged 91 days. These results disagree to the results of Ng'ambi *et al.* (2017) who observed a significant difference on digesta proventriculus pH values of male Venda chickens aged 91 days and fed dietary threonine level of 5.7 g/kg DM. According to Dibner (2004), low pH in the proventriculus is required for adequate protein digestion and general reduction in bacteria community in the different segments of the gut. Choct (2009) reported that high pH in the gut results in the proliferation of pathogenic microbiota which is detrimental to both the growth and health of chickens. However, the values in the current study are not within the range reported by Ao *et al.* (2008), who observed that gut organ pH for chickens is ranges between 5.5 and 6.5 but changes as digesta transit different segments of the GIT.

Results of the current study indicate that threonine inclusion levels had no effect on GIT, ileum, jejunum, duodenum, and caeca lengths of indigenous male Boschveld chickens aged 91 days. However dietary threonine inclusion levels had effect on large

intestine lengths of male indigenous Boschveld chickens aged 91 days. Male indigenous Boschveld chickens on diets having 7,5 g/kg threonine inclusion level had longer large intestine lengths than those on diets having 4, 8, 8,5 and 9 g of threonine/kg DM. A dietary threonine level of 6.08 g/kg DM optimized large intestine lengths of male indigenous Boschveld chickens aged 91 days. However, the results of the current study disagree with Moghaddam *et al*. (2011) who observed a significant difference in optimal gut morphology of broiler chickens aged 1 to 14 days and fed dietary threonine level of 8.7 g/kg DM. Moreover, NRC (1994) recommended a dietary threonine level of 8.0 g/kg DM for optimal gut morphology for broiler chickens aged 1 to 21 days. Jamroz (2005) posited that longer intestines are assumed to digest feed efficiently and provide a greater surface area for nutrient absorption.

Results of the current study indicated that threonine inclusion levels had no effect on gizzard, ileum, duodenum, and caeca and large intestine weights of indigenous male Boschveld chickens aged 91 days. However, threonine inclusion levels had effect on the crop and jejunum weights of indigenous Boschveld chickens. Male indigenous Boschveld chickens on diets having 7.5 g/kg threonine inclusion level had heavier crop and jejunum weights than those on diets having 4, 8, 8.5 and 9 g of threonine/kg DM. Dietary threonine levels of 5.95 and 5.66 g/kg DM, respectively optimized crop, and jejunum of male indigenous Boschveld chickens aged 91 days. However, the present results disagree to those of El-Faham *et al.* (2017) who observed significant differences in optimal gut organ weights of broiler chickens aged 21 to 42 days and fed dietary threonine level of 8.7 g/kg DM. Jamroz (2005) stated that the increase in gut organ weight allows broiler chickens to reach a heavier body weight faster compared to indigenous chickens. Rezaeipour *et al.* (2012) observed that the results of internal organ weights of broilers at the starter period were not significantly changed because of different levels of dietary threonine.

The current results indicate that threonine inclusion levels had effect on the carcass, thigh, drumstick, breast, and abdominal fats of male indigenous Boschveld chickens aged 91 days. Male indigenous Boschveld chickens on diets having 9 g/kg threonine inclusion level had heavier carcass weight than those on diets with 4, 7.5, 8 and 8.5 g of threonine/kg DM. Furthermore, the positive relationships between threonine inclusion levels and carcass weights were observed. The results of the current study

are in line with Lemme (2001) who observed significant differences in optimal carcass weight of 42 days old broiler chickens fed threonine level of 7.4 g/kg DM. NRC (1994) suggested that a threonine level of 7.4 g/kg DM optimized carcass weight of 42 days old broiler chickens. Dozier *et al.* (2000) reported that increasing dietary threonine level of 0.0011 g/kg had no significant difference in carcass yield of broiler chickens. Several reports suggest that broiler diets containing more threonine than recommended levels improve edible meat percentage.

Male indigenous Boschveld chickens on diets having 9 g/kg threonine inclusion level had heavier drumstick than those on diets having 4, 7.5, 8 and 8.5 g threonine/kg DM. Furthermore, a positive relationship between threonine inclusion levels and drumstick weight were observed. However, the results of the current study are in line with El-Faham *et al.* (2017) who observed significant differences in drumstick meat weight of broiler chickens aged 21 to 42 days and fed threonine level of 8.7 g/kg DM. Moreover, Rezaeipour *et al*., (2012) and Abbasi *et al.,* (2014) recommended lower dietary threonine levels of 7.7 and 7.9 g/kg DM respectively for optimal drumstick meat weights of broiler chickens aged 1 to 42 days.

Male indigenous Boschveld chickens on diets having 9 g/kg threonine inclusion level had heavier breast weights than those on diets having 4, 7.5, 8 and 8.5 g of threonine/kg DM. Furthermore, the positive relationships between threonine inclusion levels and breast weight were observed. The results of the current study are in line with Kidd *et al.* (2004) observed a significant difference in optimal breast meat weights of broiler chickens aged 21 to 42 days and fed threonine level of 8.7 g/kg DM. Moreover, Corzo *et al.* (2007) recommended a lower dietary threonine level of 7.9 g/kg DM for optimal breast meat weight of broiler chickens aged 42 days. Ciftci and Ceylan (2004) reported that an increase in dietary threonine content increased the breast meat yield of broiler chickens. Similarly, Dozier *et al.* (2001) found that an increase in dietary threonine level increased drumstick weight in female broiler chickens aged 42 to 56 days.

Male indigenous Boschveld chickens on diets having 8.5 g/kg threonine inclusion level had heavier thigh weight than those on diets having 4, 7.5, 8 and 9 g of threonine/kg DM. Furthermore, a positive relationship between threonine inclusion levels and thigh

meat weight were observed. However, the results of the current study disagree with Corzo *et al.* (2007) and Kidd *et al.* (2004) who observed significant differences in optimal thigh meat weights of broiler chickens aged 21 to 42 days and fed dietary threonine levels of 8.6 and 8.7 g/kg DM, respectively. Rezaeipour *et al.* (2012) indicated that NRC recommendations of 0.0012 g/kg threonine level at the starter period are enough for optimal thigh weight.

Dietary threonine levels of 6.87 g/kg DM optimized abdominal fat of male indigenous Boschveld chickens aged 91 days. However, the results of the current study are inconsistent with Moradi *et al.* (2013) who observed a significant difference in abdominal fat of broiler chickens aged 42 days and fed dietary threonine levels of 0.0011 and 0.00115 g/kg. Hosseinpour *et al.* (2012) observed that threonine levels of 0.0009 to 0.0012 g/kg relative to NRC recommendations, had no significant effect on abdominal fat of broiler chickens. Corzo *et al.* (2003; 2009) observed no significant difference in abdominal fat of broiler chickens fed dietary threonine at different levels of 5.5, 5.7, 6.1 and 6.7 g/kg DM.

The present results indicate that threonine inclusion levels had no effect on the breast pH of indigenous Boschveld aged 91 days. However, dietary threonine inclusion levels had effect on the meat colour of male indigenous Boschveld chickens aged 91 days. In disagreement with the present results, Schiavone *et al.* (2017) found no differences in the pH of the breast meat between the control and the treatment groups that were fed threonine level of 5.2 g/kg DM.

The results of the current study disagree to those of Zadeh *et al*. (2019) who reported that 35-day-old Japanese quails fed dietary threonine levels of 6.7 and 8.1 g/kg DM had no significant difference in the meat colour. According to Bovera *et al.* (2016), the absence of differences in the meat colour after cooking is very important because colour can influence the consumer acceptance of meat. The L* (lightness), a* (redness), b* (yellowness) values for all treatments are comparable with the characteristics of normal indigenous chicken meat. Normal broiler meat is described as meat with lightness values between 50 and 56, whereas dark meat will have less than 50 value of lightness and pale meat will have higher than 50 value of lightness and the lightness on the breast meat was (44–44.2), redness was (1.07–1.18),

yellowness was (0.69–0.78) and chroma was (1.91–1.92) (Van Laack *et al*., 2000; Petraccia *et al.,* 2004). According to Alaa El-Din Ahmed Bekhita (2019), colour of fresh meat and its stability can vary widely among species and cuts from the same animal due to the differences in anatomical, physiological function, and biochemical processes of the muscles where the meat is obtained from.

Results of the current study indicated that dietary threonine levels had an affect on tenderness of indigenous Boschveld chickens aged 91 days. However, threonine inclusion levels had no effect on juiciness, flavor, overall acceptability, and cooking loss of indigenous Boschveld chickens aged 91 days.

Male indigenous Boschveld chickens on diets having 9 g/kg threonine inclusion level had a higher tenderness value than those on diets having 4, 7.5, 8 and 8.5 g of threonine/kg DM. The positive relationship between threonine inclusion levels and tenderness was observed. The results of the current study are in line with Ng'ambi *et al*. (2017) who found significant differences in meat flavour, tenderness, and juiciness in indigenous Venda chickens fed dietary threonine levels of 5.977, 6.103 and 5.977 g/kg DM, respectively. In disagreement with the present results, 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. Threonine plays a major role in eliciting the characteristics of juiciness and flavour of foods (Kobayashi *et al.,* 2009). Lawrie (2006) identified three compounds (i.e., free glumatic acid, 5'-inosinic acid and potassium ion) as the taste active components in chicken meat extracts. Glutamic and 5'- inosinic acid are favourites among consumers as they constitute a characteristic taste of chicken meat (Lawrie, 2006). These findings have a lot of implications for ration formulation for slow-growing chickens.

5.2 Conclusion and recommendations

Indigenous Boschveld chickens aged 1 to 49 days

Dietary threonine levels of 4, 7.5, 8, 8.5 and 9 g/kg DM used in the current study had effect on feed intake, BWG, FCR, duodenum pH values, weights, and lengths of unsexed indigenous Boschveld chickens aged one to 49 days. However, feed intake, FCR, GIT, ileum, and jejunum lengths, duodenum pH values, and duodenum weights were optimized at different threonine levels of 12.00, 0.70 and 8.0 g/kg DM

respectively. This means dietary threonine level requirements for indigenous Boschveld chickens will depend on the production variable of interest. This has implications on ration formulation for broiler chickens. Thus, there is a need for further studies on the subject to determine the present findings.

Indigenous Boschveld chickens aged 50 to 91 days

Dietary threonine levels of 4, 7.5, 8, 8.5 and 9 g/kg DM used in the current study had effect on feed intake, BWG, FCR, large intestine lengths, crop and jejunum weights, breast meat colour, meat parts weight, tenderness, and abdominal fat of male indigenous Boschveld chickens aged 50 to 91 days. However, BWG, large intestine lengths, crop, jejunum weights, abdominal fats, and yellowness were optimized at different threonine levels of 6.12, 6.08, 5.95, 5.66 and 6.87 g/kg DM, respectively. This means that dietary threonine level requirements for indigenous Boschveld chickens will depend on the production variable of interest. Additionally, the threonine levels that optimized production parameters in this study seem to be higher than those reported in the literature. It is possible that dietary threonine requirement levels of the broiler chickens line used in the current study may be higher than for those used in the other experiments. Therefore, it is recommended that when formulating diets for indigenous Boschveld chickens, threonine levels should depend on the parameters of interest and breed as broiler chicken lines are constantly being improved through efficient breeding.

CHAPTER SIX
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. *Brazilian Journal of Poultry Science,* 16(1): 35-44.

Alaa El-Din Ahmed Bekhit, 2019. Total volatile basic nitrogen in meat products: occurrence, method of determination and use as a freshness indicator (Australian Meat Processor Corporation). *Encyclopedia of Meat Sciences*, 67: 102-118.

Amara, A. B., M. Viana da Sila, and S. C. Lannes. 2018. Lipid oxidation in meat; mechanisms and protective aactors. A Review. *Food Science and Technology Campinas*, 38: 15.

AOAC. (2012). Official Methods of Analysis, 19th edition. AOAC International, North Frederic Avenue, USA.

Ao T, 2008. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. *Poultry Science*, 88:111–117.

Aro SO, Aletor VA, Tewe OO, Fajemisin AN, Usifo B, Falowo AB (2008). Preliminary investigation on the nutrients, anti-nutrients, and mineral composition of microbially fermented cassava starch residues. Proc. 33rd Annual Conf., Nigerian Society for Animal Production (NSAP), Ayetoro, Ogun State, Nigeria: 248.25.

Atela JA, Mlambo V, and Mnisi CM, 2019. A multi-strain probiotic administered via drinking water enhances feed conversion efficiency and meat quality traits in indigenous chickens. *Animal Nutrition*, 5: 179-184.

AVMA, 2020. Guidelines for the Euthenasia of Animals. Animal Veterinary Association. Schaumburg II.

Azimu, W.; Manatbay, B.; Li, Y.; Kaimaerdan, D.; Wang, H.E.; Reheman, A.; and Muhatai, G, 2018. Genetic diversity and population structure analysis of eight local chicken breeds of Southern Xinjiang. *British Poultry Science*, 59, 629–635.

Azzam, M.M.M., Dong, X.Y., Xie, P., Wang, C and Zou, X.T. (2011). The effect of supplemental L-threonine on laying performance, serum free amino acids, and immune function of laying hens under high-temperature and high-humidity environmental climates. *Journal of Applied Poultry Research*, 20: 361–370.

Berrazaga, I., Micard, V., Gueugneau, M. and Walrand, S. 2019. The role of the anabolic properties of plant- versus animal-based protein sources in supporting muscle mass maintenance. *Journal of Animal Science*, 11(8): 1825.

Bortoluzzi, C., Rochell, S.J. and Applegate, T.J. 2018. Threonine, arginine, and glutamine: Influences on intestinal physiology, immunology, and microbiology in broilers. *Poultry Science*, 01: 1–9.

Boughter Jr., J.D., and Munger, S.D. 2013. Taste receptors. In: W. J. Lennarz and M.D. Lane, editors, Encyclopedia of Biological Chemistry. 2nd ed. Academic Press, London. p. 366–368.

Bovera, F., Loponte, R., Marono, S., Piccolo, G., Parisi, G., Iaconisi, V., Gasco, L. and Nizza, A. 2016. Use of Tenebrio molitor larvae meal as protein source in broiler diet: Effect on growth performance, nutrient digestibility, and carcass and meat traits. *Journal of Animal Science*, 94: 639–647.

Chen, Y.P., Cheng, Y.F., Li, X.H., Yang, W.L., Wen, C., Zhuang, S, and Zhou, Y.M. 2016. 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.

Chen, Y.P., Cheng, Y.F., Li, X.H., Yang, W.L., 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.

Chen, Y.P., Cheng, Y.F., Li, X.H., Yang, W.L., 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.

Chen, Y., Zhang, H., Cheng, Y., Li, Y., Wen, C. and Zhou, Y. 2018. Dietary l-threonine supplementation attenuates lipopolysaccharide-induced inflammatory responses and intestinal barrier damage of broiler chickens at an early age. *British Journal of Nutrition*, 119: 1254–1262.

Choct, M. 2009. Managing gut health through nutrition. *British Poultry Science*, 50(1): 9–15.

Çiftci, I. and Ceylan, N. (2004). Effects of dietary and crude protein on growth performance, carcass, and meat composition of broiler chickens. *British Poultry Science*, 45: 280–289.

Corzo A, Kidd MT, Dozier WA, Kerr BJ. 2009. Dietary glycine and threonine interactive effects in broilers. *Journal of Applied Poultry Research*, 18:79–84.

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

Debnath BC, Biswas P, and Roy B. 2019. The effects of supplemental threonine on performance, carcass characteristics, immune response, gut health of broilers in subtropics during pre-starter and starter period. *Journal of Animal Physiology and Animal Nutrition*, 103(1): 29–40.

Demirok, E., Veluz, G., Stuyvenberg, W.V., Castañeda, M.P., Byrd, A, and Alvarado, C.Z. 2013. Quality and safety of broiler meat in various chilling systems. *Poultry Science*, 92: 1117–1126.

Dibner, J. 2004. Organic acids: Can they replace antibiotic growth promoters. *Feed International*, 25(12): 14–16.

Dinh, T. T. N., Thu T. N, To K. V. and Schilling M. W. 2021. Fatty acid composition of meat animals as flavor precursors. *Meat Muscle Biology Journal*, 5: 1–16.

Dong, X.Y., Azzam, M.M.M. and Zou, X.T. 2017. Effects of dietary threonine supplementation on intestinal barrier function and gut microbiota of laying hens. *Poultry Science*, 96: 3654–3663.

Dozier, W.A., Moran, E.T., and E.T. Moran Jr, 2001. Response of early- and late-developing broilers to nutritionally adequate and restrictive feeding regimens during the summer. *Journal of Applied Poultry Research*, 10: 92-98.

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

El-Faham A., Ali N.G., and Abdelaziz M. (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(1): 155-167.

Elnesr, S.S., Alagawany, M., Elwan, H.A.M., Fathi, M.A. and Farag, M.R. 2020. Sodium butyrate improves the intestinal health of poultry. *Annals of Animal Science*, 20(1):1–13.

Elnesr, S.S., Ropy, A. and Abdel-Razik, A.H. 2019. Effect of dietary sodium butyrate supplementation on growth, blood biochemistry, haematology and histomorphometry of intestine and immune organs of Japanese quail. *Annals of Animal Science*, 13(6): 1234–1244.

El Masry, G., D. W. Sun, and P. Allen. 2015. Near-infrared hyperspectral imaging for predicting colour, pH, and tenderness of fresh chicken. *Journal of Food Engineering*, 110:127-140.

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*, 9: 382–386.

Fouad, A.M., Zhang, H.X., Chen, W., Xia, W.G., Ruan, D., Wang, S. and Zheng, C.T. 2017. Estimation of L-threonine requirements for Longyan laying ducks. *Asian-Australian Journal of Animal Science*, 30: 206–210.

Gómez, I., Ibañez, F.C. and Beriain, M.J. 2019. Physicochemical and sensory properties of sous vide meat and meat analogue products marinated and cooked at different temperature-time combinations. *International Journal Food Properties*, 22: 1693–1708.

Gravina, S. A., Yep, G.Y. and Khan, M. 2013. Human biology of taste. *Annals of Saudi Medical Journal*, 33: 217–222.

Hosseinpour A, Hasanabadi A, Shahir MH, Hajati H. 2012. Effect of different levels of crude protein and threonine on performance, carcass characteristics and immune system of broilers at the starter and grower periods. *Iranian Journal of Animal Science Research*. 4:191–198.

Jamroz, D. 2005. Comparative characteristic of gastrointestinal tract development and digestibility of nutrients in young chickens, ducks, and geese. Proceedings of the 15th European Symposium on Poultry Nutrition, September 25-29, Balatonfured, Hungary, pp: 74-85.

Jayasena, D.D., Ahn, D.U., Nam, K.C. and Jo, C. 2013. Factors affecting cooked chicken meat flavor: A re8view. *World's Poultry Science Journal*, 05:60-76.

Jiang Y., Tang J., Xie M., Wen Z. G., Qiao S. Y., and Hou S. S. 2017. Threonine supplementation reduces dietary protein and improves lipid metabolism in Pekin ducks. *Poultry Science*, 58: 687–693.

Kerth, C.R. and Miller, R.K. 2015. Beef flavor: a review from chemistry to consumer. *Journal of Science Food and Agriculture*, 95: 2783–2798.

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. *Italian Journal of Animal Science*, 16(4): 580-587.

Kidd, M.T. 2000. Nutritional considerations concerning threonine in broilers. World's *Poultry Science*, 56:139-151.

Kidd M.T, Corzo A., Hoehler D., Kerr B., Barber S., and Branton S. (2004). Threonine needs of broiler chickens with different growth rates. *Poultry Science*, 83(8): 1368-1375.

Kidd, M.T. and Kerr, B.J. 1996. L-Threonine for Poultry: A Review. *Journal of Applied Poultry Research*, 5: 358–367.

Kidd, M.T. and Kerr, B.J. 1997. Threonine responses in commercial broilers at 30 to 42 days. *Journal of Applied Poultry Research*, 6: 362–367.

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 Reponses. *Journal of Applied Poultry Research*, 8: 160-169.

Kim, Y.J., Lee, G.D. and Choi, I.H. (2019) Effects of dietary supplementation of red ginseng marc and alpha-tocopherol on the growth performance and meat quality of broiler chicken. *Journal of the Science of Food and Agriculture*, 94: 1816-1821.

Kralik, G., Kralik, Z., Grcevi, M. and Hanzek, D. 2018. Quality of chicken meat. In Animal Husbandry and Nutrition. https://fvhe.vfu.cz/konference/sborniky/2018-sbornik-xii.-kabrttovy-dieteticke-dny.pdf (Accessed: 2018-11-23)

Kosowska, M., Majcher, M.A., Jélen H.H. and Fortuna, T. 2018. Key aroma compounds in smoked cooked loin. *Journal of Agriculture and Food Chemistry*, 666: 3683–3690.

Kutu, F.R. and Asiwe, J.A.N. 2010. Assessment of maize and dry bean productivity under different intercrop systems and fertilization regimes. *African Journal of Agricultural Research*, 5(13): 1627-1631.

Latif, S. and Müller, J. 2015. Potential of cassava leaves in human nutrition: A review. *Trends in Food Science and Technology*, 44(2): 147-158.

Lawrie, R.A. (2006). Meat Science. 7th edition. Woodhead Publishing Limited, Cambridge, England

Lemme, A., 2001. Responses of broilers to dietary threonine: A survey of the international literature. *Amino News*, 2: 1-6.

Li X., Ha, M., Warner, R.D. and Dunshea, F.R, 2022. Collagen characteristics affect the texture of pork Longissimus and Biceps femoris. *Translational Animal Science*, 6:129.

Lisnahan, C.V. 2018. Determining the nutritional needs of native chickens in the growth phase that are reared intensively using the cafeteria method. Ph.D. Thesis, Gadjah Mada University, Indonesia

Ma, Q., Zhou, X., Sun, Y., Hu, L., Zhu, J., Shao, C., Meng, Q. and Shan, A. (2020). Threonine, but not lysine and methionine, reduces fat accumulation by regulating lipid metabolism in obese mice. *Journal of Agriculture and Food Chemistry*, 68: 4876–4883.

Maharjan P, Martinez DA, Weil J, Suesuttajit N, Umberson C, Mullenix G, Hilton KM, Beitia A and Coon CN, 2021. Physiological Growth Trend of Current Meat Broilers, and Dietary Protein and Energy Management Approaches for Sustainable Broiler Production. *African Journals*, 56: 1236-3456.

Manyelo, T.G., Selaledi, L., Hassan, Z.M. and Mabelebele, M. 2020. Local chicken breeds of Africa: Their description, uses and conservation methods. *Animals*, 10: 2257.

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

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

10.1201/9781439832271.

Melen, M., Pavelková, A., Hašèik, P., Bobko, M., and Èubon, J. (2014). Sensory evaluation of Cobb 500 chicken meat after application of different additives in their nutrition. *Postravinarstvo*, 8(1):184-189.

Min, Y., Liu, S., Qu, Z., Meng, G. and Gao, Y. 2017. Effects of dietary threonine levels on growth performance, serum biochemical indexes, antioxidant capacities, and gut morphology in broiler chickens. *Poultry Science*, 96: 1290–1297.

Mir, N.A.; Rafiq, A.; Kumar, F.; Singh, V.; and Shukla, V, 2017. Determinants of broiler chicken meat quality and factors affecting them: A review. *Journal of Food Science and Technology*, 54: 2997–3009.

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

Moradi S., Zaghari M., Shivazad M., Osfoori R., and Mardi M, 2013. The effect of increasing feeding frequency on performance, plasma hormones and metabolites, and hepatic lipid metabolism of broiler breeder hens. *Poultry Science*, 92: 1227-1237.

Moreira Filho, A.L.B., Ferket, P.R., Malheiros, R.D., Oliveira, C.J.B., Aristimunha, P. C., Wilsmann, D.E. and Givisiez, P.E.N. 2019. Enrichment of the amnion with threonine in chicken embryos affects the small intestine development, ileal gene 770 expression and performance of broilers between 1 and 21 days of age. *Poultry Science*, 98: 1363–1370.

Morgan, N. K., and M. Choct. 2016. Cassava: Nutrient composition and nutritive value in poultry diets. *Journal of Animal Nutrition*, 2: 253-261.

Mudalal, S., Lorenzi, M., Soglia, F., Cavani, C. and Petracci, M. 2015. Implications of white striping and wooden breast abnormalities on quality traits of raw and marinated chicken meat. *Journal of Animal Science*, 9: 728–734.

NAFIS, 2017. Feed and Feedings. http://www.nafis.go.ke/livestock/poultry-chicken/general-information/feeds-and-feeding/ (Access: August 14, 2017).

Najafi, R., Ahmar, R. and Tazehkand, G.N. 2017. Effect of different dietary threonine levels on optimal growth performance and intestinal morphology in 1–14 days old Ross 308 broilers. *Brazilian Journal of Poultry Science*, 03: 200-208.

Ng'ambi, J.W., 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: 150.

Novaković, S. and Tomašević, I. 2017. A comparison between Warner-Bratzler shear force measurement and texture profile analyses of meat and meat products. Conference Series Earth and Environmental Science. 85.

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

Petracci, M., Betti, M., Bianchi, M. and Cavani, C. 2004. Color variation and characterization of broiler breast meat during processing in Italy. *Poultry Science*, 83: 2086–2092.

Qaisrani S.N., van Krimpen M.M., Kwakkel R.P., Verstegen M.W.A., Hendriks W.H. (2018). Diet structure, butyric acid, and fermentable carbohydrates influence growth performance, gut morphology, and cecal fermentation characteristics in broilers. *Poultry Science*, 94: 2152–2164.

Ramos-Elorduy, J., Moreno, J. M. P., Prado, E. E., Perez, M. A., Otero, J. L., and De Guevara, O. L. (2017). Nutritional value of edible insects from the state of Oaxaca, Mexico. *Journal of Food Composition and Analysis*, 10(2): 142–157.

Ravindran, V. 2013. Monogastric Research Centre, Institute of Food, Nutrition and Human Health, Massey University, Palmerston North, New Zealand.

Rezaei, M., Nassiri, M.H., Pour, R.J. and Kermanshahi, H. 2004. The effects of dietary protein and lysine levels on broiler performance, carcass characteristics and N excretion. *International Journal of Poultry Science*, 3(2): 149-152.

Rezaeipour V, Fononi H, Irani M. 2012. Effect of dietary L-Threonine and Saccharomyces cerevisiae on performance, intestinal morphology, and immune response of broiler chickens. *South African Journal of Animal Science*. 42:266–273.

Rezaeipour, V., Valizadeh, A., Abdullahpour, R. and Sadeghi, A.R. 2015. Effects of dietary threonine and a multi strains probiotic (Primalac) supplementation on growth performance, blood metabolites and carcass characteristics in Japanese quails. *Poultry Science*, 3: 135-141.

Samadi, L.F. 2006. Modeling of threonine requirement in fast-growing chickens, depending on age, sex, protein deposition, and dietary threonine efficiency. *Poultry Science*, 85(11): 1961-1968.

SAS. 2020. Statistical Analysis System, User's Guide. Statistical. Version 9.4 th ed. SAS. Inst. Inc.

Schiavone, A.; Cullere, M.; De Marco, M.; Meneguz, M.; Biasato, I.; Bergagna, S.; Dezzutto, D.; Gai, F.; Dabbou, S.; and Gasco, L. 2017. Effect on growth performances, feed-choice, blood traits, carcass characteristics and meat quality. *Italian Journal of Animal Science*, 16: 93–100.

Selaledi L, Maake M, Mabelebele M, 2021. The acceptability of yellow mealworm as chicken feed: a case study of small-scale farmers in South Africa. *Scholarly Journal*, 10: 118- 140.

Smith, D.P., Lyon, C.E. and Lyon, B.G. 2002. The effect of age, dietary carbohydrate source, and feed withdrawal on broiler breast fillet colour. *Poultry Science*, 81:1584-1588.

Sugiharto, S., Yudiarti, T., Isroli, I., Widiastuti, E., Wahyuni, H.I. and Sartono, T.A. 2019. Recent advances in the incorporation of leaf meals in broiler diets. Livestock Research for Rural Development. *Animal Nutrition*, 5,1-10.

Russell W.M.S., and Burch R.L, 1959. The Principles of Humane Experimental Technique. Methuen & Co Ltd.; London, UK.

Thirumalaisamy J. Muralidharan S. Senthilkumar R. Hema Sa and Priyadharsini M, 2019. Cost effective feeding of poultry, *Poultry Science*, 6: 3997 – 4005.

Thomas, D. V. 2008. Nutrient digestibility and energy utilisation of diets based on wheat, sorghum, or maize by the newly hatched broiler chick. *British Poultry Science*, 49:429-435.

Van Laack, R.L.J.M., Liu, C.H., Smith, M.O. and Loveday, H.D, 2000. Characteristics of pale, soft, exudative broiler breast meat. *Poultry Science*, 79: 1057–1061.

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: 165–172.

Yasar S, and Gok MS, 2016. Fattening performance of Japanese quails (Coturnix coturnix japonica) fed on diets with high levels of dry fermented wheat, barley, and oats grains in whey with citrus pomace. *Bulletin UASVM Animal Sciences and Biotechnologies,* 71: 51–62.

Zadeh ZS, Kheiri Fand Faghan M. 2019. Productive performance, egg-related indices, blood profiles, and interferon- gene expression of laying Japanese quails fed on Tenebrio molitor larva meal as a replacement for fish meal*. Italian Journal of Animal Science,* 19: 27-24.

Zampiga, M., Flees, J. Meluzzi, A., Dridi, S. and Sirri, F. 2018. Application of omics technologies for a deeper Insight into quali-quantitative production traits in broiler chickens: A review. *Journal of Animal Science and Biotechnology*, 9: 61.

Zhang, Q., Xu, L., Doster, A., Murdoch, R., Cotter, P., Gardner, A., Applegate, T.J. 2014. Dietary threonine requirement of Pekin ducks from 15 to 35 days of age based on performance, yield, serum natural antibodies, and intestinal mucin secretion. *Poultry Science*, 93: 1972–1980.

Zimoch, J. and Findlay, C.J. 1998. Effective discrimination of meat tenderness using dual attribute time intensity. *Journal of Food Science*, 63: 940-944.