EFFECT OF DIETARY METHIONINE LEVEL ON PRODUCTIVITY AND CARCASS CHARACTERISTICS OF ROSS 308 BROILER CHICKENS

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by

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BSC AGRICULTURE (ANIMAL PRODUCTION)

A RESEARCH MINI-DISSERTATION

Submitted in fulfilment of the requirements for the degree MASTER OF SCIENCE IN

AGRICULTURE (ANIMAL PRODUCTION)

in the

FACULTY OF SCIENCE AND AGRICULTURE

(School of Agricultural and Environmental Sciences)

at the

UNIVERSITY OF LIMPOPO

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2019

DECLARATION

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

Signature………………………….. Date……………………………………

Ms Paledi Mashego Queen

ACKNOWLEDGEMENTS

I would like to acknowledge the assistance, guidance, support, encouragement and supervision accorded to me by my supervisor, Prof J.W. Ng'ambi, for his patience, vigilance, strictness and critical supervision which made this work what it is. Thank you for ensuring that I complete this dissertation successfully. To my co-supervisor, Dr D. Brown, for his guidance, assistance, time and encouragement that made me to believe in myself in everything I do. Thank you for your assistance. I would, also, like to thank the National Research Foundation (NRF) and VLIROUS scholarships for financial support during the entire period of my study.

I, also, wish to express my appreciation for the technical assistance by Mr M.M. Ginindza and Ms P Nhleko, University of Limpopo Animal Unit and Experimental farm workers, fellow MSc students (Naum Makhubela, Jamela Ngomani and Grace Manyelo). Your encouragement and support is greatly appreciated. To Cedara laboratory, thank you for helping me with my analyses.

I, also, wish to extend my special appreciations to my lovely parents (David and Lindiwe Paledi), my sisters (Pretty, Sue, Sunday, Vicky, Dolly, Refilwe and Dimpho Paledi), brother (Freedom Paledi), fiancé (Balcken Maleboho) and niece (Bokamoso Paledi) for their love, support, understanding, encouragement, tolerance and prayers during the entire period of my study.

Above all, I would like to give outmost praise and glory to the almighty Lord, who gave me strength, knowledge, wisdom, understanding and skills. Thanks to the Almighty Father for your protection, love and guidance that made me succeed.

DEDICATIONS

This dissertation is dedicated to God Almighty for His guidance and blessings throughout the study period.

To my parents Lefokisi David and Matau Lindiwe Paledi for encouraging and praying for me. I want to thank you for all your efforts.

ABSTRACT

Two experiments were conducted to determine the effect of dietary methionine level on productivity and carcass characteristics of Ross 308 broiler chickens. In each experiment, the diets were isocaloric and isonitrogenous but with different dietary methionine levels. Five diets were formulated to contain dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM. The first experiment commenced with 300 unsexed Ross 308 broiler chickens with initial average live weights of 42 ± 2g per chicken. The chickens were randomly assigned to five treatments with five replications, resulting in 25-floor pens with 12 chickens per replicate. The second experiment commenced with 150 male Ross 308 broiler chickens with initial average live weight of 637 ± 12g per chicken. The chickens were randomly assigned to five treatments with three replications, resulting in 15-floor pens with 10 chickens per replicate. A complete randomized design was used in each experiment. Data was analysed using the General Linear Model (GLM) procedures of the statistical analysis of variance, Version 9.3.1 software program. Where there were significant differences, mean separation was done using the Tukey test at the 5% level of significance. A quadratic regression model was used to determine the optimal productivity of the chickens while a linear model was used to determine the relationships between dietary methionine level and responses by the chickens in the variables measured.

The treatments for the first experiment were UM_4 (4g methionine/kg DM), UM_5 (5g methionine/kg DM), UM_6 (6g methionine/kg DM), UM_8 (8g methionine/kg DM) and UM_9 (9g methionine/kg DM). Feed intake, growth rate, feed conversion ratio (FCR), metabolisable energy intake and nitrogen retention of unsexed Ross 308 broiler chickens aged one to 21 days were not affected (P>0.05) by dietary methionine level. Similarly, dietary methionine level did not have any effect (P>0.05) on diet crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and fat digestibilities in unsexed Ross 308 broiler chickens aged 14 to 21 days. Dietary methionine level did not have any effect on live weights of broiler chickens at 21 days. Live weights of unsexed Ross 308 broiler chickens aged 7 or 14 days were not improved (P>0.05) by increasing dietary methionine level from 4 to 9g/kg DM. Crop, gizzard and small intestine weights and crop, proventriculus and gizzard digesta pH values of unsexed Ross 308 broiler chickens aged 21 days were not affected

(P>0.05) by dietary methionine level. Similarly, dietary methionine level did not improve (P>0.05) caecum and large intestine lengths of unsexed Ross 308 broiler chickens aged 21 days. However, dietary methionine level affected (P<0.05) dry matter (DM) and ash digestibilities of unsexed Ross 308 broiler chickens aged 14 to 21 days. Proventriculus and large intestine weights, gastrointestinal tract and small intestine lengths of unsexed Ross 308 broiler chickens aged 21 days were improved (P<0.05) by increasing dietary methionine level. In addition, increasing dietary methionine level increased (P<0.05) small and large intestine digesta pH values of broiler chickens aged 21 days. Thus, dry matter digestibility, live weights at day 7 ad 14, caecum length, large intestine length and digesta pH were optimized at different dietary methionine levels of 7.26, 5.29, 4.99, 6.80, 4.84 and 6.37g/kg DM feed, respectively.

The treatments for the second experiment were MM_4 (4g methionine/kg DM), MM_5 (5g methionine/kg DM), MM_6 (6g methionine/kg DM), MM_8 (8g methionine/kg DM) and MM_9 (9g methionine/kg DM). Dietary methionine level did not have effect (P>0.05) on feed intake of male Ross 308 broiler chickens aged 6 weeks. However, dietary methionine level improved (P<0.05) feed intake of male Ross 308 broiler chickens aged 4 or 5 weeks. Live weights of male Ross 308 broiler chickens aged 28 days were not affected (P>0.05) by dietary methionine level. However, live weights of male Ross 308 broiler chickens aged 35 and 42 days were affected (P<0.05) by dietary methionine level. Similarly, dietary methionine level affected (P<0.05) DM, CP, ADF, NDF, fat and ash digestibilities of male Ross 308 broiler chickens aged 35 to 42 days. Thus, dietary methionine levels of 6.93, 7.70, 6.85 and 11.27g/kg DM optimized dry matter, CP and fat digestibilities, and live weight of male broiler chickens aged 42 days.

Dietary methionine level did not affect (P>0.05) FCR, growth rate and metabolisable energy intakes of male Ross 308 broiler chickens. Increasing dietary methionine level from 4 to 9g/kg DM improved (P<0.05) nitrogen retention of male Ross 308 broiler chickens aged 22 to 42 days. Dietary methionine level did not have any effect (P>0.05) on proventriculus, gizzard, caecum and large intestine weights, caecum, small and large intestine lengths, and crop, gizzard, caecum and large intestine digesta pH values of male Ross 308 broiler chickens aged 42 days. Crop and small

intestine weights and gastrointestinal tract lengths of male Ross 308 broiler chickens aged 42 days were improved ($P<0.05$) by dietary methionine level. Similarly, dietary methionine level affected ($P<0.05$) proventriculus and small intestine digesta pH values of male Ross 308 broiler chickens aged 42 days. Thus, dietary methionine levels of 6.558 and 7.851g/kg DM optimized broiler chicken crop weight and GIT length.

Dietary methionine level affected ($P<0.05$) carcass organ weights of male Ross 308 broiler chickens aged 42 days. Increasing dietary methionine level increased chicken breast meat weight. However, there was no clear trend for the other carcass organs. Meat flavour and shear force values of male Ross 308 broiler chickens aged 42 days were not affected ($P>0.05$) by dietary methionine level. However, dietary methionine level affected meat tenderness and juiciness. Thus, dietary methionine levels of 10.09 and 13.32g/kg DM optimized broiler chicken meat tenderness and juiciness.

Key words: Dietary methionine level, Ross 308 broiler chickens, Feed intake, Growth rate, Live weights, Carcass characteristics.

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Cu	Copper
Fe	Iron
K	Potassium

LIST OF ABBREVIATIONS

ADF	Acid detergent fibre.
ANOVA	Analysis of variance
AOAC	Association of Analytical Chemists.
Ca	Calcium.
CF	Crude fibre.
cm	Centimetre.
CP	Crude protein.
Cu	Copper
^0C	Degree centigrade.
DM	Dry matter.
DMD	Dry matter digestibility
DMI	Dry matter intake.
FAO	Food and Agricultural Organisation.
FCR	Feed conversion ratio.
Fe	Iron
g	Gram.
GE	Gross energy.
GIT	Gastro-intestinal tract.
GLM	General Linear Model.
K	Potassium
kg	Kilograms.
LSD	Least significant difference.
ME	Metabolisable energy.
Mg	Magnesium
Mn	Manganese
N	Nitrogen.
Na	Sodium
NDF	Neutral detergent fibre.
NRC	National Research Council.
P	Phosphorus.
%	Percentage
r	Correlation

r^2	Coefficient of determination
SAS	Statistical Analysis System.
SEM	Standard error of the means.
WBSF	Warner-Bratzler Shear Force
Zn	Zinc

CHAPTER ONE

INTRODUCTION

1.1 Background

There has been a lot of improvements in broiler production, mainly through efficient genetic improvement (Vieira and Angel, 2012). Whilst rapid growth rate continues to be the most important selection criterion, there has been increasing interest in other genetic aspects of broiler chickens, such as improving feed conversion efficiency, reducing fat content, improving meat yield, changing the shape of the growth curve, and attempting to overcome the problems of leg weakness (Gous,1986). In addition, genetic improvements in the broiler industry have been immense and far exceed the progress made in the meat, where improvements in disease control, environmental control and nutrition (Gous, 1986). Advances in poultry nutrition are fundamental to match developments in genetic improvement (Neto *et al*., 2013). It is, therefore, important to determine the nutrient requirements of these chickens. Such data can be useful in devising strategies for improving productivity and carcass characteristics of the present broiler chickens.

1.2 Problem statement

The composition and carcass weight of broiler chickens are receiving considerable attention. There is an emphasis on improving meat production and reducing fat content of broiler chicken carcasses (Ng'ambi *et al.,* 2009). Methionine requirement levels for optimal productivity of Ross 308 broiler chickens are changing due to breed improvements. Similarly, the relationships between methionine requirement levels and total protein requirements of such improved chickens are also changing due to genetic improvement of the breed. All this requires that dietary nutrients match improved chicken requirements for maintenance and tissue accretion in order to obtain optimal productivity. Broiler chickens are not capable of synthesizing methionine *de novo* which makes it a nutritionally essential amino acid (NRC, 1994). This means that methionine requirements have to be supplied in the feeds. It is, therefore, important to determine dietary methionine levels for optimal productivity and carcass characteristics of Ross 308 broiler chickens.

1.3 Scientific contribution of the study

This study generated information on the effect of dietary methionine level on productivity and carcass characteristics of Ross 308 broiler chickens. Such information will help in devising strategies aimed at improving productivity of Ross 308 broiler chickens. Improved productivity of the chickens may entail higher economic and nutritional benefits to farmers raising Ross 308 broiler chickens.

1.4 Aim

The aim of this study was to determine dietary methionine levels for optimal productivity and carcass characteristics of Ross 308 broiler chickens, assuming all other nutrients were adequate.

1.5 Objectives

The objectives of this study were to determine:

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

1.6 Hypotheses

The hypotheses of this study were as follows:

i. Dietary methionine level has no effect on feed intake, growth rate and carcass characteristics of Ross 308 broiler chickens aged one to 42 days.
ii. There are no optimal responses in feed intake, growth rate and carcass characteristics of Ross 308 broiler chickens aged one to 42 days to dietary methionine level.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Poultry nutrition has improved a lot over the past 100 years. In spite of advances made on the nutritional aspects, nutritional problems still remain unsolved and serve as a challenge to non-ruminant nutritionists (Murawska *et al*., 2018). The role of genetics in poultry production has become the backbone in improving growth and reproductive performance as well as increasing profitability (Petracci *et al*., 2015). However, nutrition has not matched improvements due to animal breeding. Hence, reassessment of nutrient requirements is essential to bridge the gap between the genetic improvement and nutritional requirements.

Amino acids in the diets of broiler chickens are very important. Amongst all the essential amino acids required by poultry, methionine is usually the first limiting amino acid in the diets (Drazbo *et al*., 2015). Methionine deficiency is usually caused by the fact that large amounts of plant protein sources are currently being used in feeds while levels of animal and fish protein sources have been reduced (Carew *et al*., 1997; Elwert *et al*., 2008). Methionine should be supplied in broiler chicken diets, as birds are unable to synthesize it in the amounts necessary to sustain life and growth. Methionine is required at higher than normal levels to comply with the increased tissue demands when chickens grow fast (Opoola *et al*., 2016). Thus, methionine levels in the diet for optimal productivity of chickens must be determined.

2.2 Broiler chicken production

In South Africa, broiler production dominates the agricultural sector and it is the main source of animal protein followed by beef (Sonaiya, 2007; Sarkar and Bell, 2006). In developing countries, chicken production is the best alternative form of income generation, plays a significant role in family nutrition and above all, smallholder poultry provides a good opportunity to address poverty alleviation (Temesgen *et al*., 2018). It is also the cheapest and most consumed meat in the communal areas (Abbas *et al*., 2016). Broiler production focuses on growing meat birds using the fastest and most efficient method possible. Commercial and small holder producers raise broiler chickens to reach market weight in about five weeks depending on the requirements of a particular market (Neto *et al*., 2013).

Chickens of broiler strains have been selected for rapid weight gain and efficient utilization of feed. Broiler chickens are usually allowed to feed on an *ad libitum* basis to ensure rapid development to market size, although some interest has been expressed in controlling feed intake in an attempt to minimize the development of excessive carcass fat (Temesgen *et al*., 2018). Broiler chickens are marketed at a wide range of ages and body weights. Females may be grown to 900 to 1,000g body weight to supply Cornish hens, mixed sexes may be reared to 1.8 to 2 kg for use as whole birds and some parts, and males may be grown to 2.8 to 3 kg for deboned meat (Amin and Cheah, 2003). Thus, it is difficult to establish a single set of requirements that is appropriate to all types of broiler production. Nutrient requirements may vary according to the criterion of adequacy. In the instance of essential amino acids, greater dietary concentrations may be required to optimize efficiency of feed utilization than would be needed to maximize weight gain (Neto *et a*l., 2013; Applegate, 2008).

2.3 Nutritional requirements of broiler chickens

Nutritional requirements used for commercial poultry production are developed based on the recommendations of the National Research Council (NRC, 1994). The recommendations, shown in Table 1, are based on research completed before that time. Much has since changed in the genetics of chickens being grown, and the breeder companies have developed requirements specific for the genotypes they have developed. Since the NRC (1994) requirements were developed for slower growing strains of chickens, they may be more applicable to the slower-growing strains of chickens being developed for use in alternative production systems (Jacob, 2013). Few studies have compared methionine requirements between broiler strains differing in growth rates. The methionine contents given in the NRC (1994) are generally minimum levels that satisfy general productive activities of broiler chickens without strain being considered. In general, fast-growing broiler chickens have superior growth performance and breast muscle yield as compared to slow-growing broiler chickens (Wen *et al*., 2017). Thus, it can be implied that the responses of growth performance and breast muscle yield to dietary methionine status may be different between fast-growing and slow-growing broiler chickens.

Some of the factors that may affect requirements of broiler chickens include age and gender. Some studies suggest that males require larger quantities of nutrients than do females at a similar age (Pesti, 2009). However, when expressed as a percentage of the diet, there seems to be little difference in nutrient requirements of the sexes. Wen *et al*. (2017) reported that the requirements for many nutrients seem to diminish with age, but for most nutrients there have been few studies designed to precisely estimate requirements for all age periods, especially for those beyond 3 weeks of age. Relatively high concentrations of dietary amino acids are needed to support the rapid growth of meat-type chickens. Body weights of commercial meat-type chickens will increase 50 to 55-fold by 6 weeks after hatching. A large part of this increase in weight is tissue of substantial protein contents (Yaqoob and Mubarak, 2018). Thus, adequate amino acid nutrition is vital to the successful feeding program for such chickens.

Table 2.1 Broiler chicken requirements

Nutrient	Unit	Starter (0-10 days)	Grower (11-24 days)	Finisher (>25 days)
Protein	%	22-25	21-23	19-21
Metabolisable energy	MJ/kg	12.60	13.30	13.50
	Kcal/kg	3010	3175	3225
Total Arginine	%	1.48	1.31	1.11
Digestible Arginine	%	1.33	1.18	1.00
Total Lysine	%	1.44	1.25	1.05
Digestible Lysine	%	1.27	1.10	0.92
Total Methionine	%	0.51	0.45	0.39
Digestible Methionine	%	0.47	0.42	0.36
Total Methionine +Cystine	%	1.09	0.97	0.83
Digestible Methionine +Cystine	%	0.94	0.84	0.72
Total Threonine	%	0.93	0.82	0.71
Digestible Threonine	%	0.80	0.70	0.61
Total Trypophan	%	0.25	0.22	0.19
Digestible Tryptophan	%	0.22	0.19	0.17
Total Valine	%	1.09	0.96	0.81
Digestible Valine	%	0.94	0.83	0.70
Calcium	%	1.0	0.90	0.85
Av.phosphorous	%	0.50	0.45	0.42
Sodium	%	0.16	0.16	0.16

Source: NRC (1994)

2.4 Production of methionine

Animals are unable to synthesise methionine in their bodies. Methionine (Figure 2.1) must be supplemented or supplied in the feeds according to the animal's requirement (Vieira *et al*., 2004). In plants and microbes, methionine can be made through a process called methionine biosynthesis. This process starts with aspartic acid. Aspartic acid is reduced into homoserine. Homoserine then becomes active using a phosphate molecule. The hydroxyl or -OH active group is then replaced by another amino acid like cysteine or a derivative of methionine. This allows the sulfur molecule to bind and the molecules go through methylation, with the addition of CH^3 (Jacob, 2013).

Methionine additives used in poultry and other feeds are L-methionine, DL-methionine or liquid methionine hydroxy-analogue free acid (MHA-FA) which is the hydroxyl analogue of methionine (Golshahi *et al*., 2013). All plants and animals can only utilise L-isomer methionine and only L-type methionine present in proteins. D-isomer of methionine and MHA can also be used, but it must be converted into the L-form through enzyme reaction in the body (Drażbo *et al*., 2015). Meat-chickens in the grower and finisher phases can obtain sufficient methionine while foraging pastures (Moritz *et al*., 2005). According to Jacob (2013), this would include the plant material consumed as well as any insects they can catch. However, obtaining significant methionine from pasture depends highly on forage composition and management and environmental conditions. It may be more useful for small flocks than for large-scale organic poultry production (Buchanan *et al*., 2007).

Methionine requirements of poultry can be met without the use of synthetic methionine. This includes alternative ingredients as well as natural sources of methionine (Applegate, 2008). The challenge of organic producers is to meet the methionine requirement of poultry without the use of synthetic methionine. Animal protein such as fish meal is high in methionine, but producers who feed a vegetarian diet do not include any animal products (Wen *et al*., 2017). Excessive fish meal can also give poultry products a fishy taste, and there are sustainability issues with overfishing (Jacob, 2013). Corn gluten meal is another possible methionine-rich feed ingredient. However, organically produced corn gluten meal is not available. Sesame hulls, a byproduct of sesame paste production, have been reported to be high in methionine (Farran *et al*., 2000). It's recommended use, however, is limited to 8% in broiler starter diets and 14% in layer diets. Sesame paste (also known as tahini) is widely used in cooking in many countries, and availability of the hulls may be higher in countries where it is produced (Farran *et al*., 2000).

2.5 Biochemical functions of methionine

Methionine is considered the first sulfur-containing amino acid which is vital for the health and productivity of poultry and is important for different functions in the bird's body (Shini *et al*., 2005). Methionine is, therefore, a key dietary nutrient for maintaining growth and production of growing chicks in the broiler industry (Applegate, 2008; Garlich, 1985). This amino acid has at least four main roles in broiler chickens. Firstly, methionine is a glutathione precursor, participates in protein synthesis, a tripeptide that reduces reactive oxygen species and thus protects cells from oxidative stress. It is, also, required for the synthesis of polyamines (spermine and spermidine), which take part in nucleus and cell division events. Lastly, methionine is the most important methyl group donor for methylation reactions of DNA and other molecules (Rubin *et al*., 2007). This amino acid provides methyl groups, which are needed for several metabolic reactions such as the synthesis of carnithine and creatine (Ahmed and Abbas, 2011). Co-enzyme S-adenosyl methionine serves as an important labile methyl-group donor, which allows for the formation of many essential compounds in the chicken's body including choline, creatine, epinephrine, DNA or glutathione, which are major sources of organic sulfur compounds in the body (Rubin *et al*., 2007).

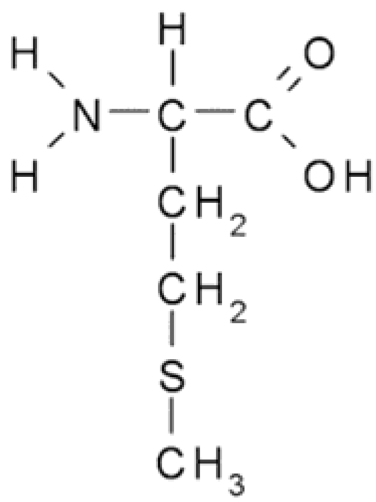

Figure 2.1 Structure of methionine

2.6 Methionine requirements of broiler chickens

Protein is the building block of amino acids (El-Wahab *et al*., 2015). Once consumed, proteins are broken down into amino acids, and they are then absorbed by the animal to produce the specific proteins that they require (El-Wahab *et al*., 2015). In poultry, 22 amino acids are needed to form body protein, among them, 10 are indispensable for monogastric animal production and the remaining are dispensable (Baker, 2009; Wu, 2009). Some of the amino acids can be synthesized by the bird (dispensable), whereas others cannot be made at all to meet metabolic needs (indispensable). Sufficient amount of dispensable amino acids must be supplied to prevent the conversion of essential amino acids into non-essential amino acids (Belloir *et al*., 2015).

Indispensable amino acids such as methionine, threonine and lysine cannot be produced by chickens. These essential amino acids must, therefore, be fed in order to supply the building blocks needed in the synthesis of body proteins (Mcdonald *et al*., 2010). All these amino acids play an important role for optimal growth and meat yield of broiler chickens (Shini *et al*., 2005). Additionally, if the amino acids supplied are not in the proper, or ideal, ratio in relation to the needs of the chickens, then amino acids in excess of the least limiting amino acid will be deaminated and likely used as a source of energy rather than towards body protein synthesis (Applegate, 2008).

Methionine and lysine are usually the most important limiting amino acids in poultry nutrition and are frequently supplemented in the formulated diets (Gill, 2003). There are a number of studies that have been conducted to determine the requirements of methionine and lysine as the first two limiting amino acids in practical corn-soybean based diets for broiler chickens. Si *et al*. (2001) suggested that levels of methionine and lysine in excess of NRC (1994) recommendations may result in enhanced performance, weight gain and feed conversion ratio. Murray *et al*. (1998) also found that addition of synthetic amino acids like lysine and methionine at high levels to the diet can stimulate insulin secretion from pancrease by aggregating plasma which in turn releases amino acids and fatty acids from the body reserves and leads to protein synthesis. However, Bouyeh and Gevorgyan (2011) observed that highest level of lysine and methionine (40% more than NRC) had lowest body weight gain in comparison with diets supplemented with 10 or 30% more than NRC recommendations. However, there was linear decrease of feed conversion ratio (FCR) with increased supplementation of lysine and methionine. The requirements for methionine by poultry at various phases of growth have not been adequately assessed in recent years. It is likely that such requirements would have changed as bird genotypes change (Denner and Bessi, 2003). Nutrient requirements need to be updated with the constant improvement in performance and carcass yield of commercial broiler chickens breeding.

National Research Council (1994) estimated that the total dietary methionine requirement for broiler chickens aged 21 to 42 days is 7.4g/kg DM while for those aged 42 to 56 days is 6.8g/kg DM. Higher amounts of dietary methionine levels of 6.7 and 7.5g /kg DM are recommended for optimal growth rates of broiler chickens aged 6 to 7 and 4 to 6 weeks, respectively (Ng'ambi *et al.,* 2009). NRC (1994) estimated that the total dietary methionine requirement for broiler chickens aged 6 to 8 weeks is 0.68%. Meirelles *et al*. (2003) indicated that dietary methionine requirement of male broiler chickens aged 6 to 8 weeks is 0.60% for optimal live weight. Chickens that are given a diet containing less than the required amount of methionine levels have poor feed conversion ratio (FRC), poor growth rate and lower carcass yield (Choawit and Chaiyapoom, 2009).

The information on the effect of dietary methionine level on feed intake, growth rate, and mortality, and carcass characteristics of Ross 308 broiler chickens is, therefore, inconclusive. The discrepancies in the methionine requirements for broiler chickens may be due to breed improvements resulting in higher methionine requirements. Thus, it is important to determine dietary methionine levels for optimal productivity and carcass characteristics of Ross 308 broiler chickens.

2.7 Effects of dietary methionine on productivity and meat quality of broiler chickens

Modern broiler chickens grow very fast due to genetic selection and reach a market weight at six weeks of age with high breast meat yields (Fanatico *et al*., 2007).

However, such selection may have negative effects on the sensory and functional qualities of broiler meat (Dransfield and Sosnicki, 1999). Although consumers are accustomed to paying low prices for poultry meat, they are increasingly interested in products with good quality. Meat quality is a complex trait that is influenced by genetic, environmental and nutritional factors (Fletcher, 2002). Slow-growing chickens are generally believed to have higher meat quality than fast-growing chickens (Guan *et al*., 2013; Sarsenbek *et al*., 2013). Many studies have been conducted to evaluate the effects of nutrient density on meat quality in fast- and slow-growing chickens (Fanatico *et al*., 2007; Zhao *et al*., 2009; Yalcin *et al*., 2010; Wang *et al*., 2013). Zhao *et al*. (2012) reported that the meat quality of broiler chickens from different breeds had some different responses to dietary nutrient density. This observation may be due to the methionine influencing lipid metabolism and oxidative status (Nukreaw *et al*., 2011; Chen *et al*., 2013). Meat quality is associated with its oxidative stability, which can be reflected by total antioxidant capacity (T-AOC), activities of superoxide dismutase (SOD) and glutathione peroxidase (GPX) and levels of malondialdehyde (MDA), etc. (Jiang *et al*., 2009). The T-AOC reflects cumulative action of all the antioxidants (Ghiselli *et al*., 2000), and both SOD and GPX are endogenous antioxidant enzymes to inhibit lipid oxidation, which can be evaluated by MDA levels (Chan *et al*., 1994; Castellini *et al*., 2006). Dietary methionine status affects meat quality and antioxidant systems in fast-growing broiler chickens (Chen *et al*., 2013; Del Vesco *et al*., 2015; Conde-Aguilera *et al*., 2016). According to Dozier *et al*. (2008), fast and slow-growing broiler chickens may have different methionine requirements. Thus, they may have different responses of meat quality and oxidative status to dietary methionine status (Chen *et al*., 2013).

Duclos *et al*. (2007) suggested that the rapid growth and increased breast muscle development of conventional broiler chickens was related to larger muscle fiber size and lower fat deposition, which could negatively influence meat quality. These researchers also reported that slower-growing poultry had higher levels of muscle glycogen stores and higher heminic pigments that yielded meat with a lower ultimate pH and a darker, more uniform appearance (Duclos *et al*., 2007). Other studies show that meat from slow-growing broiler chickens have poor water-holding capacity (Fanatico *et al*., 2007) and higher cook loss (Fanatico *et al*., 2005) than meat from faster-growing conventional broiler chickens. Fanatico *et al*. (2007) found a difference in the protein and fat content for meat obtained from slow-growing broiler chickens with outdoor access (13.9% protein, 4.5% fat) and fast-growing broiler chickens grown indoors (13% protein, 8.9% fat) and suggested a relationship between meat fat content and water-holding capacity or cook loss. These studies indicate differences in meat quality among genotypes with different growth rates and different housing systems.

The carcass yield of commercial broiler chickens is an important factor in the poultry industry (Amin and Mercier, 2014). There is an emphasis on increasing the meat yield, particularly breast meat and decreasing the fat content of carcass. An important aspect of the protein and methionine interrelationship is the ability of both to act as lipotrophic agents (Kalbande *et al*., 2009). Requirement for methionine can largely be related to existing dietary protein (Opoola *et al*., 2016). This was suggested after a series of experiments in which protein and methionine levels were altered for broiler chickens. Morris *et al.* (1992) concluded that the amount of methionine need to be optimized since performance increased with increase in protein and methionine. However, the reduction in abdominal fat occurred in response to increased dietary methionine and protein. According to Ng'ambi *et al*. (2009) crude protein and amino acid status of a diet can influence the carcass composition of broiler chickens, with increased carcass protein and reduced carcass fat accompanying increases in dietary protein or essential amino acid contents. Mirzaaghatabar *et al*. (2011) indicated that dietary amino acid deficiency reduces concentrations of most amino acids in plasma and destroys the immune system. Methionine deficiency in broiler chickens leads to poor productivity in terms of reduced weight gain, feed efficiency and protein content in the carcass (Opoola *et al*., 2016). On the other hand, feeding excess dietary methionine has been reported to impair body weight gain (Wang *et al*., 2004), although Han and Baker (1994) reported that 0.5 % excesses of methionine are not harmful to broiler chicks fed corn-soybean meal diets. Thus, adequate methionine levels are needed to support optimal growth rates, carcass characteristics and reduced mortality rates (Mohamed and Talha, 2011). Methionine requirements in broiler chickens depend on several factors like age, sex and genotype (Denner and Bessi, 2003). Broiler chickens need to be supplied with sufficient nutrients to meet its requirements for maintenance and growth of all components of the bird, including feathers (Opoola *et al*., 2016). It is important to determine for optimal productivity of Ross 308 broiler chickens.

Methionine affects growth and breast meat yield in broiler chickens (Mohsen *et al*., 2012). It is further reported that dietary methionine increases muscle mass, feed efficiency, carcass yield, breast meat yield, feather development and live weight gain of heat-stressed broiler chickens (Kidd *et al*., 2000). Saki *et al*. (2007) noted that an addition of methionine reduces the abdominal fat of broiler chickens. It is evident that feeding broiler chickens with increasing concentrations of methionine leads to a decrease in abdominal fat and an increase in growth rate, breast muscle yield, and leg muscle yield (Wallis, 1999; Mandal *et al*., 2004; Liu *et al*., 2006). Additionally, methionine uptake and utilization may vary in relation to the source of methionine used (Richards *et al*., 2005; Sangali *et al*., 2015). The authors further stated that only 0.025g of methionine per kg DM diet is needed for maximum growth and feed efficiency.

Different methionine supplementation levels affect the carcass characteristics in broiler chickens. Halder and Roy (2007) found higher carcass percentage in broiler chickens fed diets containing methionine levels of 0.24 and 0.29% during starter and finisher phases, respectively. Ojano-Dirain and Waldroup (2002) reported positive relationships between dressing percentage and level of methionine used. Rodrigueiro *et al*. (2000) conducted research on male and female broiler chickens separately and the results showed higher carcass percentage and breast yield at dietary methionine levels of 0.69 and 0.93% for male and female broiler chickens, respectively. The increase in the methionine level in diet increased the thigh percentage (Halder and Roy, 2007) but abdominal fat percentage was decreased (Halder and Roy, 2007; Oliveira *et al*., 2007; Kalinowski *et al*., 2003; Rezaeipour *et al*., 2012). Relative weight of liver also improved by feeding higher dietary methionine level to broiler chickens (Yaqoob and Mubarak, 2018). Adequate dietary level of methionine is needed to support optimum growth and carcass yield of fast-growing commercial broilers (Ojano-Dirain and Waldroup, 2002).

Saki *et al*. (2007) found that percentage of breast meat yield in large white toms increased by diets formulated to provide 85 to 120% of NRC (1994) amino acid recommendations. Morris *et al*. (1992) suggested that for the purpose of formulation of practical diets, minimum methionine concentration should be specified as a proportion of the protein rather than as a proportion of the diet. The authors indicated that for an estimate of maximum growth or feed efficiency, 0.025 g methionine/kg CP could be useful (Saki *et al*., 2007). Kidd *et al*. (1997) noted that similar trends were found in growth and feed conversion ratio by decreasing CP to 92% of NRC (1994) recommendations and supplementation of lysine, methionine, theronine and tryptophan (at 105% NRC level) compared with control diet. Sell (1993) found that reduction in breast meat yield occurred by reducing CP protein diet to 93% of NRC (1994) recommendations and supplementing with lysine and methionine. Body weight and feed conversion ratio were not affected by these diets. Otherwise, in a number of other studies, breast meat yield was not affected by protein level (Saki *et al*., 2007).

According to Drażbo *et al*. (2015), dietary supplementation with methionine increases feed utilisation and, thus, improves performance. In the study of fast-growing turkeys, conducted by Kubińska *et al*. (2014), the authors demonstrated that immune system function can be modulated by increasing dietary methionine concentrations. However, if the dietary supply of methionine is low, the body synthesises adequate quantities of the semi-essential amino acid cysteine from methionine and both amino acids participate in protein synthesis (Gardzielewska *et al*., 2005). According to some authors, methionine as well as methionine + cysteine levels in poultry diets are too low (Wallis, 1999; Café and Waldroup, 2006). Studies have shown that a dietary methionine deficiency significantly inhibits broiler growth, compared to a deficiency of other essential amino acids (Carew *et al*., 1997; Elwert *et al*., 2008). According to Hesabi *et al.* (2006) and Cengiz *et al*. (2008), excessive methionine intake decreases feed intake, and thus reduces the body weight gains of chickens. Thus, adequate dietary methionine levels are required to increase lean carcass content and reduce abdominal fat deposition in broiler chickens (Liu *et al*., 2010).

Many studies have been conducted to evaluate the effect of different levels (starter phase 0.31-1.10%; finisher phase 0.29-0.87%) of methionine on growth performance of broiler chickens. Results of these studies showed that increasing dietary methionine level increased the body weight gain. Rubin *et al*. (2017) observed better FCR at a methionine level of 0.51% compared with 0.31% methionine in the diet of broiler chickens. Halder and Roy (2007) also found better FCR at 0.54% methionine in starter diets compared to a 0.42% methionine level. Dietary methionine level, also, affects the feed efficiency. Yaqoob Mubarak (2018) reported that FCR decreased by increasing the methionine level from 0.50 to 0.56%. Yaqoob and Mubarak (2018) conducted two experiments on broiler chickens during starter phase and got lowest FCR at dietary methionine level of 1.10%. Best FCR was found at 0.41% dietary methionine while the results of another experiment showed better FCR at 0.51% methionine in a diet (Yaqoob and Mubarak, 2018). Differences in methionine requirements might be due to different strains of broiler chickens.

Methionine affects the immune system, improving both cellular and humoral immune response. It was reported that methionine requirements for optimal immunity are higher than for optimal growth (Tsiagbe *et al*., 1987; Swain and Johri, 2000; Shini *et al*., 2005), and that restriction of sulfur amino acids (SAA) results in severe lymphocyte depletion in intestinal tissues and lamina propria (Swain and Johri, 2000). Reports indicate that the NRC (1994) recommendations of 0.5% and 0.38% methionine for the age of 0 to 3 weeks and 3 to 6 weeks respectively, are too low for obtaining maximum broiler chicken performance due to changes in genetic, nutrition and management of broiler chickens (Hickling *et al*., 1990; Takahashi and Akiba, 1995; Kalinowski *et al*., 2003). There is evidence that essential amino acid levels in the feed higher than those of NRC (1994) specifications are needed to achieve optimal immune systems and growth performance, and to compensate for the depressed growth performance in hot conditions (Quentin *et al.*, 2005). Infections leading to several changes in amino acid plasma levels and dietary levels of certain individual amino acids have been shown to affect immune response (Quentin *et al.*, 2005).

2.8 Effects of dietary methionine on sensory attributes of broiler chicken meat

Palatability is defined by flavour, tenderness and juiciness. In the past, meat quality was more closely related to the sensory perceptions, freshness, and safety aspects, whereas more recently it is associated with nutrition, well-being and functionality in relation to human health (Petracci *et al*., 2015). Nevertheless, sensory quality is crucial for consumer acceptance (Mir *et al*., 2017). Dietary supplementation is the key factor which can most easily be manipulated and has one of the most profound effects on sensory quality of meat (Joo *et al*., 2013). When evaluating the sensory attributes of products, such as appearance, odour, flavour, taste and texture, consumers respond based on their perceptions (Chumngoen and Tan, 2015). By using sensory analysis, producers can identify and respond to consumer preferences more efficiently than by using the instruments, thus increasing their competitiveness and segmenting their specific market (Sow and Grongnet, 2010).

Tenderness has been noted as the most important factor in consumer perception of palatability or quality of meat products (Anadon 2002). Therefore, this attribute has drawn the most attention from researchers. Broiler chicken production at mass level has already been achieved and now emphasis is being laid on increasing meat quality by altering various characteristics of broiler meat. Appearance, texture, juiciness, wateriness, firmness, tenderness, odour and flavour are the most important and perceptible meat features that influence the initial and final quality judgment by consumers before and after purchasing a meat product (Mir *et al*., 2017). The quantifiable properties of meat such as water holding capacity, shear force, drip loss, cook loss, pH, shelf life, collagen content, protein solubility, cohesiveness, and fat binding capacity are indispensable for processors involved in the manufacture of value-added meat products. Nutrition of birds has a significant impact on poultry meat quality and safety (Northcutt, 2009). It is well known that dietary fatty acid profiles are reflected in tissue fatty acid. Management of poultry meat production is reflected mostly on consumption features (juiciness, tenderness, flavour) of meat (Petracci *et al*., 2015).

Flavour is another quality attribute that consumers use to determine the acceptability of poultry meat (Mir *et al*., 2017). Both taste and odour contribute to the flavour of poultry meat. Flavour development occurs while cooking of poultry meat due to sugar and amino acid interactions, lipid and thermal oxidation and thiamin degradation (Mir *et al*., 2017). These chemical changes are not unique to poultry but the lipids and fats in poultry are unique and combine with odour to account for the characteristic 'poultry' flavour (Northcutt, 2009). Generally, it is not only difficult to produce a flavour defect but also to enhance flavour during production and processing. Breed or strain variation in palatability of the meat is well documented. For instance, Hinai-jidori chickens of Japan (Kiyohara *et al.,* 2011), Korean native or farm chickens (Jung *et al.,* 2011) and kadaknath of India have significantly higher flavour scores compared to that of broiler chickens (Mir *et al*., 2017). The reason for breed variation may be due to variations in content of inosine-50-monophosphate (IMP) (Tang *et al.,* 2009), arachidonic acid and docosahexaenoic acid (DHA) and amino acids, including aspartic acid, threonine, serine, glycine, alanine, tyrosine, lysine, and arginine (Lee *et al*., 2012).

The selection practised by commercial broiler breeders for traits such as fast growth rate and increased breast muscle yield have often been assumed to negatively impact on the eating quality of broiler meat and on skeletal and cardiovascular well-being of the live bird (Sandercock *et al*., 2009). Sandercock *et al*. (2009) showed that genetic variation for appearance traits was moderately high and suggested that differences seen were most likely the result of selection for broiler chicken traits. The slower growth rate and higher activity of indigenous chickens may contribute to differences in the properties of their meats (Petracci *et al*., 2015). Slower-growing chickens have been shown to be more popular as a result of a firmer texture and more intense flavour (Castellini *et al*., 2008). This can be seen in the systems employed in rearing French Label Rouge hybrids. Due to a slower growth rate and largely cereal based diet they have been shown to have 10% more muscle development, resulting in a firmer textured and darker coloured meat with more desirable flavour, and a roughly 15% decrease in both inter- and intra-muscular fat (Petracci *et al*., 2015). The differences seen in meat quality between indigenous chickens and conventional broiler chickens are predominantly related to colour, flavour, and texture (Mir *et al*., 2017).

The transformation of muscle into meat after slaughter is characterised by *rigor mortis* and the pH changes within the muscle, and their ultimate effect on meat quality (Guerrero-Legarreta, 2010). Meat quality in general is considered an extremely complex topic that can be approached from different points of view. When evaluating meat quality it is important to assess carcass conformation characteristics as well as good aesthetic, sensory and nutritional characteristics (Bogosavljevic-Boskovic *et al*., 2010). Although substantial progress has been made in broiler chicken growth and efficiency of growth, a general failure to include selection for meat quality parameters has resulted in the appearance of abnormalities in meat products such as pale, soft and exudative meat and dark, firm, and dry meat (Souza *et al*., 2011).

2.9 Conclusion

Broiler chickens play an important role in the households as a source of income and nutrition. It is the cheapest and most consumed meat among South African households. Due to genetic improvements of broiler chickens, nutrient requirements of Ross 308 broiler chickens keep on changing. Several studies showed that inadequate methionine level in the diet may lead to poor productivity and mortality. which may affect profits and the economy of the country. Information on the effect of dietary methionine level on feed intake, digestibility, growth rate and mortality, and carcass characteristics of broiler chickens is inconclusive. It is, therefore, important to ascertain the effect of dietary methionine level on productivity and carcass characteristics of Ross 308 broiler chickens.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Two experiments were conducted at the University of Limpopo Livestock unit, Limpopo Province, South Africa. The University of Limpopo lies at latitude 27.55˚S and longitude 24.77˚E. The mean ambient temperatures around the study area are 28˚C during winter and 36˚C during summer (Shiringani, 2007).

3.2 Preparation of the house

The poultry house was thoroughly cleaned with water and then disinfected using Vet GL 20 disinfectant (NTK, Polokwane). Drinkers and feeders were cleaned and disinfected with Vet GL 20 before use. The house was left empty for 7 days prior to the start of the experiment in order to eliminate the population of infectious microorganisms. The experimental house was divided into 15-floor pens of $2m^2$ per pen. Fresh sawdust was spread in each pen to a thickness of 8cm high. The heating of the house was done using 250 watt-infrared lights.

3.3 Acquisition of materials and chickens

Ross 308 broiler chicks were acquired from Lufafa Hatchery, Tzaneen, South Africa. Commercial grower mash was acquired from Voorslagvoere Milling Company at Mokopane, South Africa. Bovine colostrum was obtained from the Limpopo Dairy, situated in Louis Trichardt, South Africa. Vet GL 20 disinfectant, 250 watts infrared lights, feeders and drinkers were acquired from NTK, Polokwane, South Africa.

3.4 Experimental designs, treatments and procedures

The first part of the study (1-21 days old chickens) commenced with 300 day-old unsexed Ross 308 broiler chicks with an initial live weight of 40 ± 2g/bird. The chicks were assigned to isocaloric (16.2MJ of energy/kg DM) and isonitrogenous (220g CP/kg DM) diets in a completely randomised design. The dietary treatments had different levels of methionine, indicated as UM_4 (4g methionine/kg DM), UM_5 (5g methionine/kg DM), UM_6 (6g methionine/kg DM), UM_8 (8g methionine/kg DM) or UM_9 (9g methionine/kg DM).The treatments are in Table 3.1. Feed compositions of the diets are indicated in Table 3.2. Each treatment had five replicates, with 12 chickens per replicate. The chickens were offered feed and water *ad libitum*. Light was provided 24 hours per day (natural light during the day and artificial light during the night).

The second part of the study (22-42 days old chickens) determined the effect of dietary methionine level on the productivity and carcass characteristics of male Ross 308 broiler chickens. A total of 150 chickens, weighing an average of 637 ±12.0g each, were used in a completely randomised design (SAS, 2008) having five treatments, replicated three times with ten chickens per replicate. The diets were isocaloric (12MJ/kg) and isonitrogenenous (200g CP/kg DM). The dietary treatments had different levels of methionine indicated as MM_4 (4g methionine/kg DM), MM_5 (5g methionine/kg DM), MM_6 (6g methionine/kg DM), MM_8 (8g methionine/kg DM) or MM_9 (9g methionine/kg DM). The diets and diet compositions are presented in Tables 3.3 and 3.4, respectively. Prior to the start of this experiment, the chickens were fed a diet containing 220g CP/kg DM and 12MJ/kg DM, meeting their nutritional requirements according to NRC (1994). The chickens were offered feed and water *ad libitum*. Light was provided 24 hours per day. Sick chickens were isolated and slaughtered accordingly. Dead chickens were burnt according to University of Limpopo Animal Research Ethics Committee (UL AREC) regulations. A post-mortem was conducted by a registered veterinarian to determine the cause of sickness or death of the chickens. At 42 days of age, all the remaining chickens per pen were slaughtered humanely according to UL AREC protocol.

Table 3.1 Treatments for broiler chickens aged one to 21 days

Diet Code	Diet description
UM_4	Unsexed Ross 308 broiler chickens on a 22% CP diet having 4g of methionine/kg DM
UM_5	Unsexed Ross 308 broiler chickens on a 22% CP diet having 5g of methionine/kg DM
UM_6	Unsexed Ross 308 broiler chickens on a 22% CP diet having 6g of methionine/kg DM
UM_8	Unsexed Ross 308 broiler chickens on a 22% CP diet having 8g of methionine/kg DM
UM_9	Unsexed Ross 308 broiler chickens on a 22% CP diet having 9g of methionine/kg DM

3.5 Data Collection

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

Apparent digestibility was determined when the chickens were aged 14 to 21 days and 35 to 42 days. Apparent digestibility trials were conducted in specially designed metabolic cages equipped with separate feed and water troughs. One bird was randomly selected from each replicate and transferred to the metabolic cage for the measurement of apparent digestibility. A three-day acclimatization period was allowed prior to a four-day total faecal collection period. Droppings voided by each bird were collected daily at 8:00 hours.

Chickens were slaughtered at the ages of 21 and 42 days in accordance with the guidelines of the University of Limpopo Animal Research Ethics Committee to determine gut organ weights and lengths and gut organ digesta pH. Before the slaughtering, each chicken was weighed using an electronic weighing balance. The carcasses were then put inside a bucket containing hot water for a few seconds and they were then taken out. The carcasses were then put on a table for defeathering with hands. The carcasses were cut open at the abdominal site and the digestive tracts were removed from the abdominal cavities of the chickens. After slaughter, the carcass weight of each chicken was measured only at the age of 42 days. Gastrointestinal tract, small intestine, caeca and large intestine lengths were determined using a tape measure. The pH of gut contents (crop, proventriculus, gizzard, ileum, caecum, and colon) were measured using a digital pH meter (Crison, Basic 20 pH meter). Breast, drumstick, thigh, crop, proventriculus, gizzard, small intestine, caeca and large intestine weights were measured using an electronic weighing balance. The effect of dietary methionine on meat shear force value was determined using a texture analyser equipped with a Warmer-Bratzler shear force apparatus (American Meat Science Association, 1995).

3.6 Sensory evaluation

Meat samples which were previously frozen at -40°C for 4 days were thawed for 7 hours at room temperature prior to cooking. The breast meat was prepared and the skin was left on the meat samples. The method adopted by Pavelková *et al.* (2013) was used for sensory evaluation of the meat. The following sensory attributes were evaluated by the sensory panel: tenderness, juiciness and flavour of meat samples. The sensory panel consisted of 20 trained panellists. Each panellist was offered to drink lemon juice after tasting meat from each treatment before proceeding to the next treatment as to wash out the previous treatment to avoid confusion of tastes. The five-point ranking scale scores used are as indicated in Table 3.5. Nothing was added to the meat samples so as not to affect taste. An oven set at 105ºC was allowed to preheat prior to cooking. The meat samples were put in trays and they were covered with aluminium foil to prevent water loss. Thereafter, the trays with meat were put in an oven for approximately 60 minutes and the meat samples were turned after every 10 minutes. Samples were cut into small 5cm cubic pieces and served immediately after cooking. The individual breast meat was selected for sensory evaluation because of ease of handling.

Table 3.2 Diet composition of starter diets for Ross 308 broiler chickens

	Treatment				
	UM_4	UM_5	UM_6	UM_8	UM_9
Feed ingredients					
Maize (%)	54.49	54.70	54.92	55.32	55.53
Maize gluten 60 (%)	5.00	5.00	5.00	5.00	5.00
Wheat bran (%)	4.06	3.99	3.92	3.79	3.72
Soybean 46 (%)	29.33	29.09	28.85	28.39	28.15
L- lysine HCL (%)	0.10	0.10	0.10	0.10	0.10
DL methionine (%)	0.03	0.14	0.24	0.45	0.55
L- threonine (%)	0.05	0.05	0.05	0.05	0.05
Vit+min premix (%)	0.10	0.10	0.10	0.10	0.10
Limestone (%)	1.78	1.78	1.78	1.78	1.79
Salt (%)	0.50	0.50	0.50	0.50	0.50
Monocalcium phosphate (%)	1.57	1.58	1.58	1.58	1.58
Sodium bicarbonate (%)	0.30	0.30	0.30	0.30	0.30
Oil – Sunflower (%)	2.69	2.67	2.60	2.64	2.63
Total	100	100	100	100	100
Nutrients					
Crude protein (%)	22	22	22	22	22
Energy (MJ/kg DM)	16.2	16.2	16.2	16.2	16.2
Ash (%)	7.01	7.25	6.77	6.90	6.56
Fat (%)	3.48	4.39	4.20	3.47	4.22
ADF (%)	5.69	5.26	5.35	6.55	4.99
NDF (%)	15.03	17.36	14.51	15.80	13.88
Ca (%)	1.03	1.04	1.03	1.03	1.03

Table 3.3 Treatments for broiler chickens aged 22 to 42 days

Diet Code	Diet description
MM_4	Male Ross 308 broiler chickens on a 20% CP diet having 4g of methionine/kg DM
MM_5	Male Ross 308 broiler chickens on a 20% CP diet having 5g of methionine/kg DM
MM_6	Male Ross 308 broiler chickens on a 20% CP diet having 6g of methionine/kg DM
MM_8	Male Ross 308 broiler chickens on a 20% CP diet having 8g of methionine/kg DM
MM_9	Male Ross 308 broiler chickens on a 20% CP diet having 9g of methionine/kg DM

3.7 Shear force

Shear force assessment was done according to Warner-Bratzler Shear Force determination procedures (Dawson *et al.*, 1991). Frozen samples of chicken breast meat were thawed for 24h at 2°C. The samples were removed, tagged and used for cooked Warner Bratzler Shear Force (WBSF) measurements. Cooked meat was prepared by boiling breast cuts in a cylindrical pot using an electric stove. An electric Astove was set on for 25 min prior to preparation. The cuts were boiled to an internal temperature of 35°C, then turned and finished at 70°C. Cooked cuts were cooled down to room temperature (18°C) for at least 2 hours before WBSF measurements. Three cylindrical samples (12.5 mm core diameter) of each cut were cored parallel to the grain of the meat and sheared perpendicular to the fibre direction using a Warner-Bratzler shear device mounted on a Universal Instron Apparatus (cross head speed = 200 mm / min, one shear in the centre of each core). The reported value in kg represents the average of three peak force measurements of each sample.

Table 3.4 Diet composition of grower diets for Ross 308 broiler chickens

	Treatment				
	MM_4	MM_5	MM_6	MM_8	MM_9
Feed ingredients					
Maize (%)	55.00	55.00	55.00	55.00	55.00
Maize gluten 60 (%)	1.99	1.70	1.42	0.87	0.60
Wheat bran (%)	2.56	2.47	2.38	2.20	1.77
Soybean 46 (%)	29.35	29.54	29.73	30.09	30.37
L- lysine HCL (%)	0.10	0.10	0.10	0.10	0.10
DL methionine (%)	0.08	0.19	0.29	0.50	0.61
L- threonine (%)	0.05	0.05	0.05	0.05	0.05
Vit+min premix (%)	0.10	0.10	0.10	0.10	0.10
Limestone (%)	1.77	1.76	1.76	1.76	1.75
Salt (%)	0.50	0.50	0.50	0.50	0.50
Monocalcium phosphate (%)	1.62	1.63	1.63	1.63	1.64
Sodium bicarbonate (%)	0.30	0.30	0.30	0.30	0.50
Oil – Sunflower (%)	6.58	6.66	6.74	6.90	7.01
Total	100	100	100	100	100
Nutrients					
Crude protein (%)	20	20	20	20	20
Energy (MJ/kg DM)	16	16	16	16	16
Ash (%)	6.95	7.02	7.17	7.42	6.53
Fat (%)	3.66	5.38	6.22	6.55	4.40
ADF (%)	5.63	5.38	5.68	6.21	4.26
NDF (%)	22.18	18.06	14.73	19.84	19.68
Ca (%)	1.03	1.03	1.04	1.03	1.01

Table 3.5 Evaluation scores used by the sensory panel

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

Source: Pavelková *et al*. (2013)

3.8 Chemical analysis

Dry matter of feeds, feed refusals, faeces and meat samples were determined by drying the samples in the oven for 24 hours at the temperature of 105˚C (AOAC, 2012). Neutral and acid detergent fibre contents of feed and faeces were determined according to Van Soet *et al*. (1991). Ash contents of the feeds, faeces, feed refusals and meat samples were analysed by ashing the sample at 600˚C in a muffle furnace overnight. Ash was analysed for calcium, magnesium, phosphorous, potassium, sodium, zinc, iron, copper and manganese (AOAC, 2012). Nitrogen content of the samples was determined by Kjeldahl method (AOAC, 2010). Gross energy values of feeds, feed refusals and faeces were determined using a bomb calorimeter (AOAC, 2010). Amino acid and fatty acid contents of the diets and meat were analysed by ion-exchange chromatography at the University of Limpopo (HPLC, University of Limpopo). A full analysis for faeces and feeds was performed at the Pietermaritzburg laboratory, Kwa-Zulu Natal, South Africa according to AOAC (2000). Metabolisable energy (ME) of the diets was calculated according to AOAC (2000). Crude fat of the diet was determined following the methods of AOAC (2000).

3.9 Statistical analysis

Data on feed intake, live weight, growth rate, digestibility, feed conversion ratio, metabolisable energy, gastrointestinal morphology, shear force, sensory evaluation and carcass characteristics of male Ross 308 broiler chickens was analysed using General Linear Model (GLM) procedures of the statistical analysis of variance Version 9.3.1 software program (SAS, 2008) to detect dietary treatment effects. Where significant differences were observed, the mean separation was done using the Turkey test at the 5% level of significance (SAS, 2008). The model $y_{ij} = \mu + T_i + e_{ij}$, was applied, where $Y_{ij} = j^{th}$ is the observation of the i^{th} treatment level; μ= the overall mean; T_i= the effect of the i^{th} treatment level and e_{ij}= random error.

The responses in optimal dry matter digestibility, live weight, caecum length, large intestine length, large intestine digesta pH, crude protein digestibility, fat digestibility, crop weight, gastrointestinal tract, tenderness and juiciness to dietary methionine level were modelled using the following quadratic equation (SAS, 2008):

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

Where y = optimal dry matter digestibility, live weight, caecum and large intestine length, large intestine digesta pH, crude protein and fat digestibility, crop weight, gastro intestinal tract, tenderness and juiciness; a = intercept; b = coefficients of the quadratic equation; × = dietary methionine level and $-b_1/2b_2$ = × value for optimal response and e is the error.

The relationship between optimal responses in proventriculus digesta pH and breast weight were modelled using a linear regression equation in the form of:

$$Y = a + bx$$

Where y = proventriculus digesta pH and breast weight; a = intercept; b = coefficient of the linear equation, × = dietary methionine level.

CHAPTER 4

RESULTS

4.1 Nutrient composition of the starter diets

Results of the nutrient composition of the starter diets are presented in Table 4.1. The diets had similar protein and energy contents of 22% and 12MJ/kg DM, respectively. However, the diets had different dietary methionine levels of 0.4, 0.5, 0.6, 0.8 and 0.9% or 4, 5, 6, 8 and 9g/kg DM.

Table 4.1 Starter diet compositions (% except MJ/kg DM for energy and ppm for Zn, Cu, Mn and Fe)

	Treatment #				
Nutrient	UM_4	UM_5	UM_6	UM_8	UM_9
DM	88.57	88.57	88.58	88.58	88.58
CP	22	22	22	22	22
Energy	16.2	16.2	16.2	16.2	16.2
ADF	5.78	5.77	6.12	6.31	6.20
NDF	15.03	17.36	14.51	15.80	13.88
Fat	3.48	4.39	4.20	3.47	4.22
Ash	7.01	7.25	6.77	6.90	6.56
Ca	1.03	1.04	1.03	1.03	1.03
Mg	0.22	0.24	0.25	0.22	0.23
K	0.97	1.08	1.05	0.96	0.99
Na	0.22	0.34	0.30	0.22	0.25
K/Ca+Mg	0.40	0.40	0.37	0.37	0.37
P	0.75	0.74	0.84	0.78	0.80
Zn	43	51	47	43	43
Cu	9	1	9	7	8
Mn	75	72	77	65	66
Fe	301	271	356	293	304
Methionine level	0.4	0.5	0.6	0.8	0.9

: Treatment codes are explained in Chapter 3, Table 3.1 (The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed). There were three replicates/sample

4.2 Unsexed broiler chickens aged one to 21 days

Results of the effects of methionine level on feed intake, digestibility, growth rate, feed conversion ratio (FCR), live weight, metabolisable energy intake and nitrogen retention of unsexed Ross 308 broiler chickens aged one to 21 days are presented in Table 4.2. Dietary methionine level had no effect (P>0.05) on feed intake, growth rate, FCR, metabolisable energy intake and nitrogen retention of unsexed Ross 308 broiler chickens aged one to 21 days.

Dietary methionine level had no effect (P>0.05) on diet CP, NDF, ADF and fat digestibilities (Table 4.2). Unsexed Ross 308 broiler chickens on a diet containing 8g of methionine per kg DM had higher (P<0.05) digestibility values than those on a diet containing 4g of methionine per kg DM. However, unsexed broiler chickens on diets containing 4, 5, 6 or 9g of methionine per kg DM had the similar (P>0.05) digestibility values. Similarly, unsexed broiler chickens on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same (P>0.05) digestibility values. Unsexed broiler chickens on a diet containing 9g of methionine per kg DM had higher (P<0.05) ash digestibility values than those on diets having 5, 6 or 8g of methionine per kg DM. Similarly, chickens on a diet containing 8g of methionine per kg DM had higher (P<0.05) ash digestibility values than those containing 6g of methionine per kg DM (Table 4.2). However, unsexed broiler chickens containing 4 or 9g of methionine per kg DM had similar (P>0.05) ash digestibility values. Similarly, unsexed broiler chickens on diets containing 5, 6 or 8 had the same (P>0.05) ash digestibility values.

Live weights of unsexed Ross 308 broiler chickens aged 7 days were affected (P<0.05) by dietary methionine level (Table 4.2). Unsexed broiler chickens on diets containing 6g of methionine per kg DM had heavier (P<0.05) live weights than those on diets having 8 or 9g of methionine per kg DM. Similarly, unsexed broiler chickens on diets containing 5g of methionine per kg DM had higher (P<0.05) live weights than those having 8g of methionine per kg DM. However, unsexed broiler chickens on diets containing 4, 5 or 6g of methionine per kg DM had similar (P>0.05) live weights. Similarly, unsexed broiler chickens on diets containing 8 or 9g of methionine per kg DM had the same (P>0.05) live weights at the age of 7 days.

Dietary methionine level affected ($P<0.05$) live weights of unsexed Ross 308 broiler chickens aged 14 days (Table 4.2). Unsexed broiler chickens on diets containing 5 or 6g of methionine per kg DM had higher ($P<0.05$) live weights than those having 8 or 9g of methionine per kg DM. However, unsexed broiler chickens on diets containing 4, 5 or 6 had similar ($P>0.05$) live weights. Similarly, unsexed broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had the same ($P>0.05$) live weights. Dietary methionine level had no effect ($P>0.05$) on live weights of unsexed broiler chickens aged 21 days.

Dry matter digestibility during Week 3 and live weights of broiler chickens aged 7 or 14 days were optimized at dietary methionine levels of 7.260 ($r^2 = 0.725$), 5.294 ($r^2 = 0.752$) and 4.989 ($r^2 = 0.841$) g/kg DM, respectively (Figures 4.1, 4.2 and 4.3, respectively.

Results of the effects of methionine level on gut organ weight, length and digesta pH of unsexed Ross 308 broiler chickens aged one to 21 days are presented in Table 4.3. Dietary methionine level had no effect ($P>0.05$) on crop, gizzard and small intestine weights of unsexed Ross 308 broiler chickens aged one to 21 days. However, dietary methionine level had effect ($P<0.05$) on proventriculus weights of the chickens. Unsexed Ross 308 broiler chickens containing 5g of methionine per kg DM had higher ($P<0.05$) proventriculus weights than those on diets containing 6g of methionine per kg DM. However, chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had similar ($P>0.05$) proventriculus weights. Similarly, unsexed broiler chickens on diets containing 4, 6, 8 or 9g of methionine per kg DM had the same ($P>0.05$) proventriculus weights. Dietary methionine level affected ($P<0.05$) caecum weights of unsexed Ross 308 broiler chickens (Table 4.3). Unsexed Ross 308 broiler chickens on a diet containing 4 or 6g of methionine per kg DM had higher ($P<0.05$) caecum weights than those on diets containing 5, 8 or 9g of methionine per kg DM. However, unsexed broiler chickens containing 4 or 6g of methionine per kg DM had similar ($P>0.05$) caecum weights. Similarly, unsexed broiler chickens on diets containing 5, 8 or 9g of methionine per kg DM had the same ($P>0.05$) caecum weights.

Unsexed Ross 308 broiler chickens containing 4g of methionine per kg DM had higher (P<0.05) large intestine weights than those on a diet containing 8g of methionine per kg DM (Table 4.3). However, unsexed broiler chickens containing 4, 5, 6 or 8g of methionine per kg DM had similar (P>0.05) large intestine weights. Similarly, unsexed broiler chickens on diets containing 4, 6 or 9g of methionine per kg DM had the same (P>0.05) large intestine weight values.

Results of the present study indicate that methionine level affected (P<0.05) gastrointestinal tract, small intestine, caecum and large intestine lengths of unsexed Ross 308 broiler chickens aged one to 21 days. Unsexed broiler chickens on diets containing 5 or 6g of methionine per kg DM had longer (P<0.05) GIT than those having 8g of methionine per kg DM. However, unsexed Ross 308 broiler chickens on diets containing 4, 5, 6 or 9 had similar (P>0.05) GIT lengths. Similarly, unsexed broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had the same (P>0.05) GIT lengths. Unsexed broiler chickens on diets containing 5 or 6g of methionine per kg DM had longer (P<0.05) small intestines than those on a diet having 8g of methionine per kg DM. However, unsexed broiler chickens on diets containing 4, 5, 6 or 9g of methionine per kg DM had similar (P>0.05) small intestine lengths. Similarly, unsexed broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had the same (P>0.05) small intestine lengths.

Caecum lengths of unsexed Ross 308 broiler chickens aged 21 days were affected (P<0.05) by dietary methionine levels (Table 4.3). Unsexed broiler chickens on diets containing 6g of methionine per kg DM had longer (P<0.05) caeca than those on diets having 4 or 8g of methionine per kg DM. Similarly, unsexed broiler chickens on diets containing 5 or 9g of methionine per kg had longer (P<0.05) caecum lengths than those on diets having 4g of methionine per kg DM. However, unsexed Ross 308 broiler chickens on diets containing 4 or 8 had similar (P>0.05) caecum lengths. Similarly, unsexed broiler chickens on diets containing 5, 6 or 9g of methionine per kg DM had the same (P>0.05) caecum lengths.

Dietary methionine level affected (P<0.05) large intestine lengths of unsexed Ross 308 broiler chickens aged 21 days (Table 4.3). Unsexed Ross 308 broiler chickens on diets containing 5g of methionine per kg DM had longer (P<0.05) large intestines than those having 8 or 9g of methionine per kg DM diets. Similarly, unsexed broiler chickens on a diet containing 4 or 6g of methionine per kg had longer ($P<0.05$) large intestine lengths than those on a diet having 9g of methionine per kg DM. However, unsexed broiler chickens on diets containing 4, 5 or 6 had similar ($P>0.05$) large intestine lengths. Similarly, unsexed broiler chickens on diets containing 8 or 9g of methionine per kg DM had the same ($P>0.05$) large intestine lengths.

Dietary methionine level had no effect ($P>0.05$) on crop, proventriculus and gizzard digesta pH values of unsexed Ross 308 broiler chickens aged 21 days (Table 4.3). Unsexed Ross 308 broiler chickens on diets containing 4g of methionine per kg DM had higher ($P<0.05$) small intestine digesta pH values than those having 5 or 6g of methionine per kg DM diets. However, unsexed Ross 308 broiler chickens on diets containing 4, 8 or 9g of methionine per kg had similar ($P>0.05$) small intestine digesta pH values. Similarly, unsexed broiler chickens on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same ($P>0.05$) small intestine digesta pH values.

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

	Treatment #				
Variable	Met_4	Met_5	Met_6	Met_8	Met_9
DM Intake (g DM/chicken/day)					
Week 1	15^{a}±0.3	14^{a}±1.0	14^{a}±1.0	13^{a}±1.0	13^{a}±1.7
Week 2	46^{a}±1.3	47^{a}±1.5	46^{a}±1.9	47^{a}±1.5	46^{a}±2.0
Week 3	79^{a}±1.8	77^{a}±0.5	78^{a}±1.1	75^{a}±3.2	78^{a}±1.5
Digestibility Week 3(%)					
DM	88.0^{b}±1.18	90.8^{ab}±0.27	90.1^{ab}±1.08	92.4^{a}±1.19	90.3^{ab}±1.68
CP	71.23^{a}±4.68	66.96^{a}±8.83	70.51^{a}±6.48	70.29^{a}±9.77	73.92^{a}±5.14
NDF	26.1^{a}±10.39	31.6^{a}±5.16	28.9^{a}±7.89	41.6^{a}±10.92	40.3^{a}±9.23
ADF	32.9^{a}±7.99	33.18^{a}±4.39	27.7^{a}±8.24	36.4^{a}±9.29	36.8^{a}±5.72
Fat	79.5^{a}±5.30	85.8^{a}±3.91	82.9^{a}±2.80	80.2^{a}±6.78	87.0^{a}±2.89
Ash	46.0^{ab}±7.64	39.5^{cd}±18.26	34.2^{d}±12.21	41.7^{bc}±18.8	48.0^{a}±11.01
Growth rate (g/chicken/day)					
Week 1	12^{a}±0.7	12^{a}±0.7	12^{a}±0.8	11^{a}±0.8	11^{a}±1.2
Week 2	32^{a}±1.1	32^{a}±1.3	32^{a}±1.4	30^{a}±1.0	30^{a}±1.2
Week 3	43^{a}±3.6	46^{a}±1.1	47^{a}±3.5	47^{a}±2.4	48^{a}±1.8
FCR (g feed intake/weight gain)					
Week 1	1.3^{a}±0.06	1.2^{a}±0.06	1.2^{a}±0.02	1.2^{a}±0.07	1.2^{a}±0.04
Week 2	1.4^{a}±0.01	1.5^{a}±0.02	1.4^{a}±0.01	1.5^{a}±0.01	1.6^{a}±0.13
Week 3	1.8^{a}±0.13	1.7^{a}±0.05	1.7^{a}±0.11	1.6^{a}±0.03	1.6^{a}±0.03
Live weight (g/chicken)					
Day 7	124^{ab}±5.0	126^{ab}±4.9	128^{a}±5.6	117^{c}±4.7	118^{bc}±8.7
Day 14	347^{ab}±12.2	350^{a}±13.2	353^{a}±15.5	329^{b}±11.7	329^{b}±17.1
Day 21	650^{a}±27.5	675^{a}±8.1	678^{a}±39.8	655^{a}±28.3	666^{a}±21.4
ME (MJ/bird/day)	10.4^{a}±0.19	10.6^{a}±0.30	10.5^{a}±0.27	11.1^{a}±0.03	10.6^{a}±0.28
N-re (g/bird/day)	4.5^{a}±0.11	5.2^{a}±1.30	4.8^{a}±0.48	5.8^{a}±1.19	6.0^{a}±1.34

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

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

: Treatment codes are explained in Chapter 3, Table 3.1 (The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed).

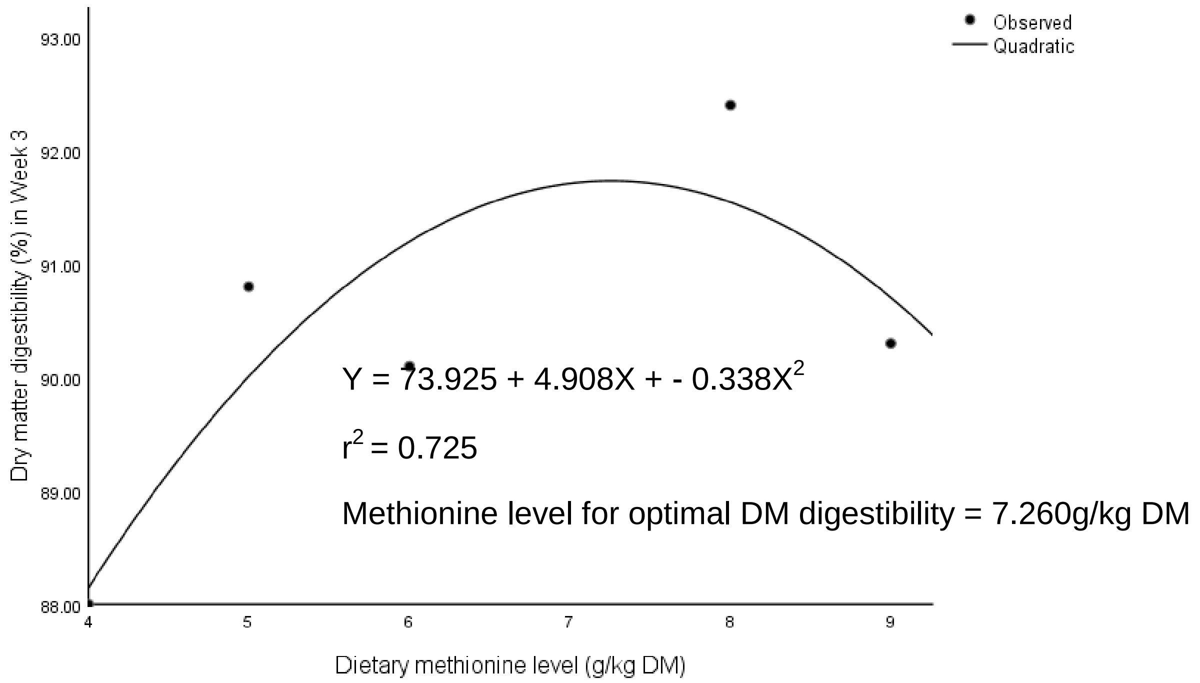

Figure 4.1 Effect of dietary methionine level on DM digestibility of unsexed Ross 308 broiler chickens aged 3 weeks

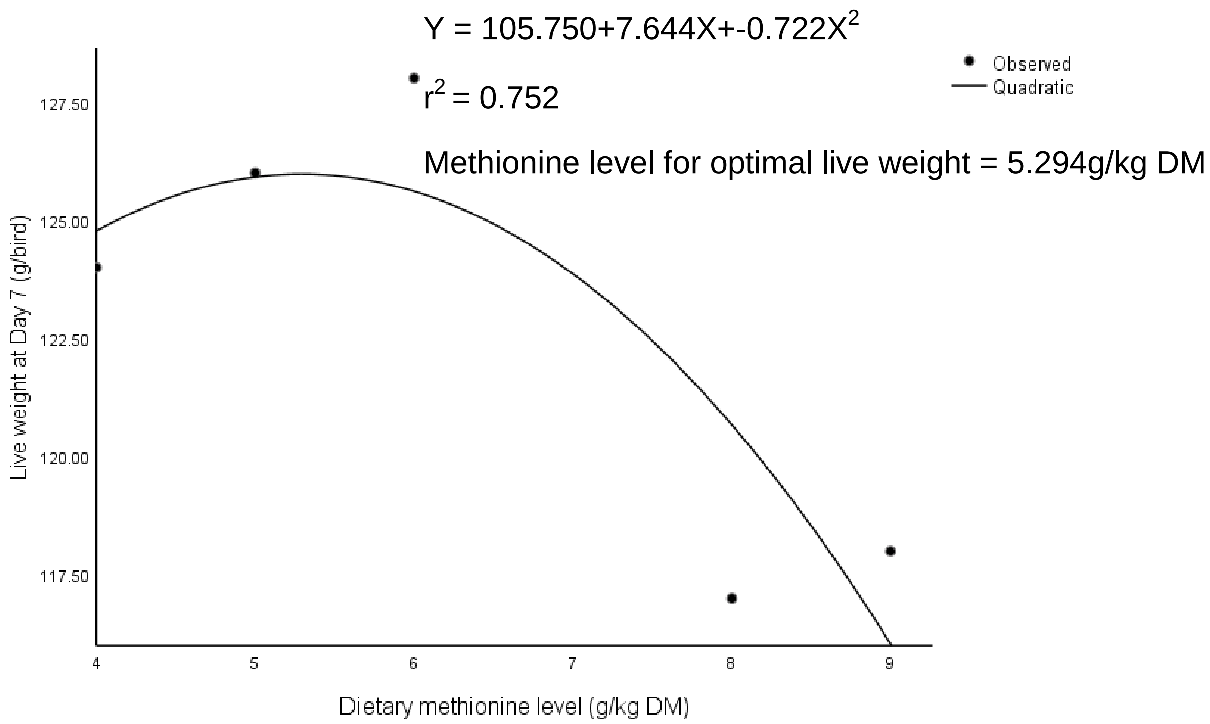

Figure 4.2 Effect of dietary methionine level on live weight of unsexed Ross 308 broiler chickens aged 7 days

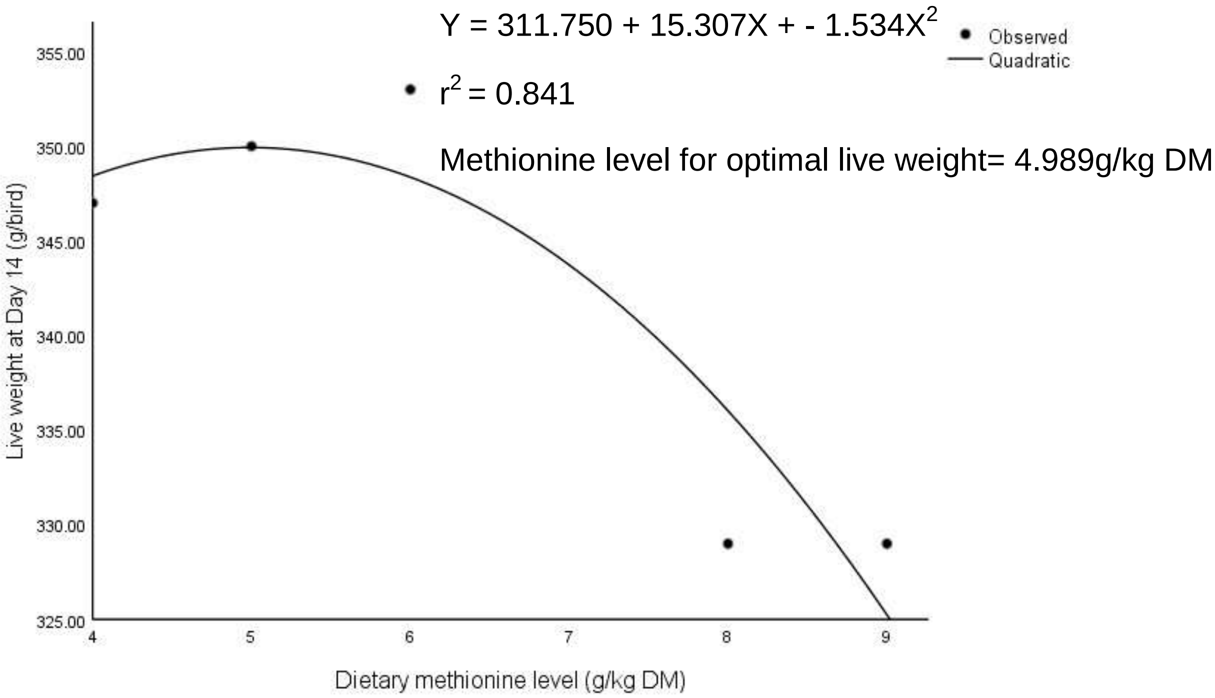

Figure 4.3 Effect of dietary methionine level on live weight of unsexed Ross 308 broiler chickens aged 14 days

Caeca of unsexed Ross 308 broiler chickens aged 21 days were affected (P<0.05) by dietary methionine level. Unsexed broiler chickens on diets containing 4 or 8g of methionine per kg DM had higher (P<0.05) caecum digesta pH values than those having 6 or 9g of methionine per kg DM. However, unsexed Ross 308 broiler chickens on diets containing 4, 5 or 8 had similar (P>0.05) caecum digesta pH values. Similarly, unsexed Ross 308 broiler chickens on diets containing 5, 6 or 9g of methionine per kg DM had the same (P>0.05) caecum digesta pH values.

Results of the present study indicate that dietary methionine level affected (P<0.05) large intestine digesta pH values of unsexed Ross 308 broiler chickens aged 21 days (Table 4.3). Unsexed broiler chickens on diets containing 9g of methionine per kg DM had higher (P<0.05) large intestine digesta pH values than those on diets having 5 or 8g of methionine per kg DM. However, unsexed Ross 308 broiler chickens on diets containing 4, 5, 6 or 8g of methionine per kg DM had similar (P>0.05) large intestine digesta pH values. Similarly, unsexed Ross 308 broiler chickens on diets containing 4, 6 or 9g of methionine per kg DM had the same (P>0.05) large intestine digesta pH values.

Table 4.3 Effect of dietary methionine level on gut organ digesta pH, length and weight of unsexed Ross 308 broiler chickens aged 21 days*

	Treatment #				
Variable	Met_4	Met_5	Met_6	Met_8	Met_9
Gut organ weight (g)					
Crop	3.2^{a}±0.37	3.5^{a}±0.37	2.5^{a}±0.55	3.6^{a}±0.42	3.3^{a}±0.32
Proventriculus	4.3^{ab}±0.35	5.0^{a}±0.15	3.6^{b}±0.27	4.4^{ab}±0.47	4.5^{ab}±0.35
Gizzard	15.4^{a}±0.41	11.5^{a}±0.73	13.3^{a}±1.59	12.6^{a}±1.25	14.1^{a}±0.15
Small intestine	40.0^{a}±7.87	43.9^{a}±2.20	47.1^{a}±7.11	34.7^{a}±3.18	38.4^{a}±0.81
Caecum	2.3^{a}±0.18	1.2^{b}±0.06	1.4^{ab}±0.52	1.7^{b}±0.29	1.3^{b}±0.15
Large intestine	3.3^{a}±0.78	1.9^{ab}±0.37	1.8^{ab}±0.42	1.4^{b}±0.23	2.1^{ab}±0.47
Gut organ length (cm)					
GIT	150^{ab}±3.2	163^{a}±2.2	161^{a}±8.3	140^{b}±5.6	156^{ab}±4.2
Small intestine	130^{ab}±1.7	139^{a}±1.7	141^{a}±8.2	122^{b}±6.2	136^{ab}±4.5
Caecum	13^{c}±0.5	14^{ab}±0.4	16^{a}±0.9	14^{bc}±0.3	14^{ab}±0.4
Large intestine	10^{ab}±0.2	11^{a}±0.6	10^{ab}±0.2	9^{bc}±0.8	8^{c}±0.4
Gut organ digesta pH					
Crop	4.4^{a}±0.16	4.2^{a}±0.03	4.3^{a}±0.21	4.6^{a}±0.68	4.2^{a}±0.25
Proventriculus	4.2^{a}±0.48	4.0^{a}±0.33	4.0^{a}±0.08	3.9^{a}±0.24	3.5^{a}±0.30
Gizzard	2.6^{a}±0.25	2.5^{a}±0.13	2.7^{a}±0.23	2.1^{a}±0.15	2.3^{a}±0.28
Small intestine	5.7^{a}±0.21	5.0^{b}±0.29	5.0^{b}±0.27	5.6^{ab}±0.18	5.4^{ab}±0.13
Caecum	6.2^{a}±0.08	6.1^{ab}±0.13	5.8^{b}±0.11	6.4^{a}±0.06	5.7^{b}±0.18
Large intestine	5.6^{ab}±0.36	5.0^{b}±0.02	5.3^{ab}±0.15	5.0^{b}±0.15	5.8^{a}±0.31

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

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

\# : Treatment codes are explained in Chapter 3, Table 3.1 (The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed)

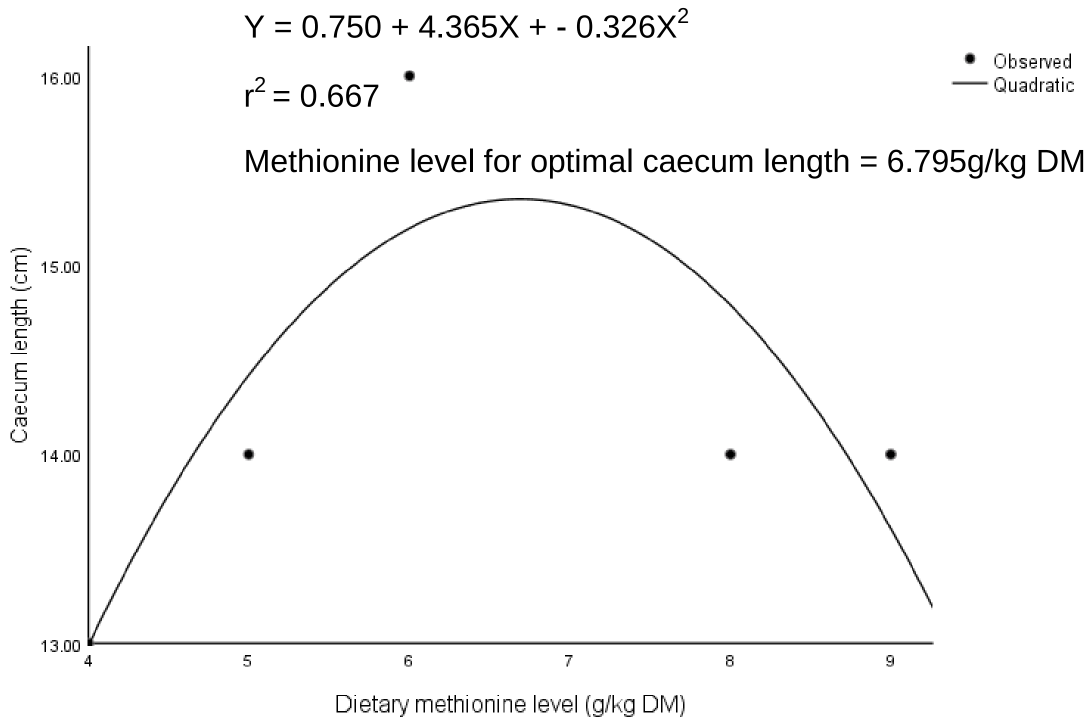

Figure 4.4 Effect of dietary methionine level on caecum length of unsexed Ross 308 broiler chickens aged 21 days

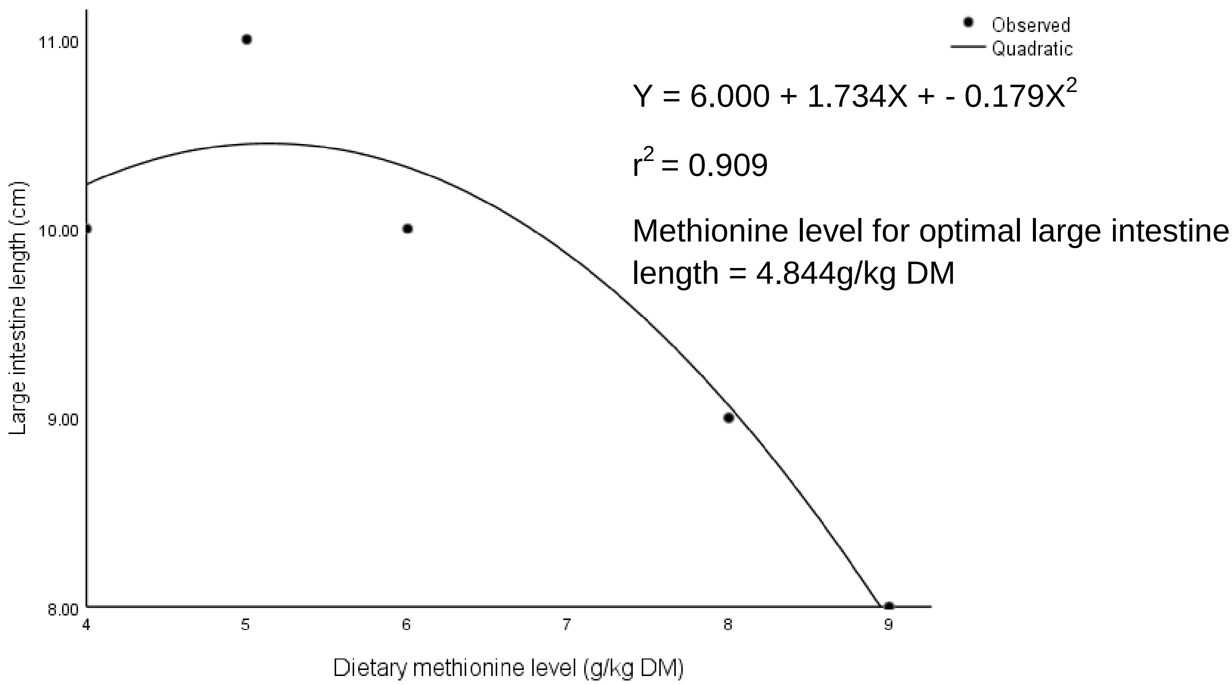

Figure 4.5 Effect of dietary methionine level on large intestine length of unsexed Ross 308 broiler chickens aged 21 days

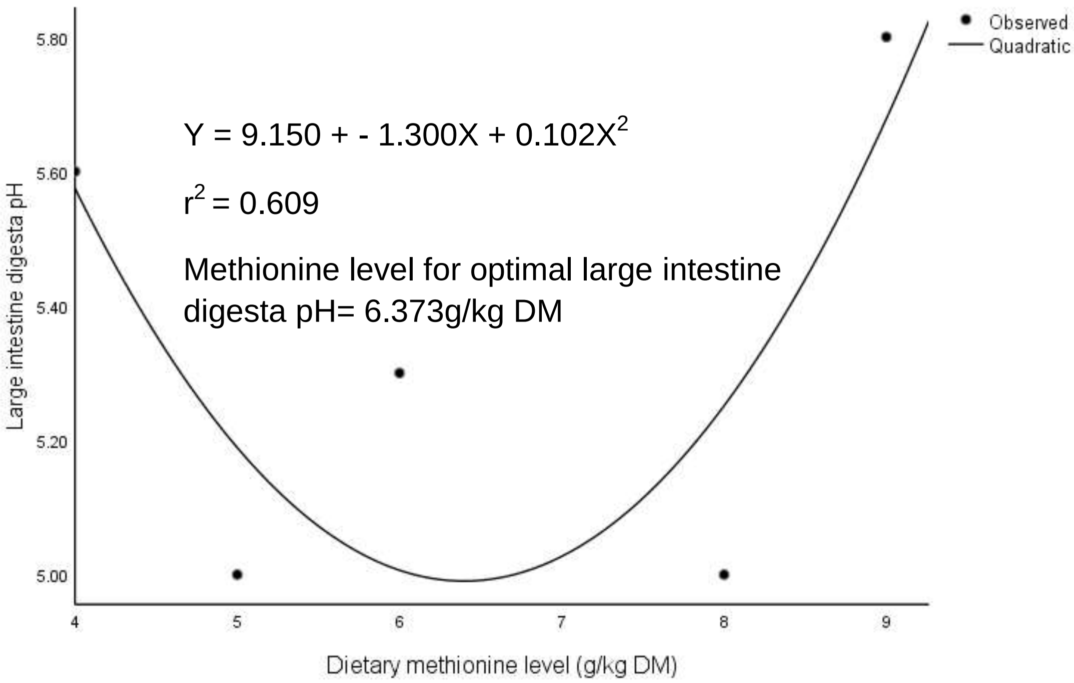

Figure 4.6 Effect of dietary methionine level on large intestine digesta pH of unsexed Ross 308 broiler chickens aged 21 days

Caecum length, large intestine length and large intestine digesta pH values of unsexesd Ross 308 broiler chickens aged 21 days were optimized at dietary methionine levels of 6.795 (r^2 = 0.667), 4.844 (r^2 = 0.909) and 6.373 (r^2 = 0.609) g/kg DM, respectively (Figures 4.4, 4.5 and 4.6, respectively)

4.3 Nutrient composition of the grower diets

Results of the nutrient composition of the grower diets are presented in Table 4.4. The diets had similar protein and energy contents of 20% and 12MJ/kg DM, respectively. However, the diets had different dietary methionine levels of 0.4, 0.5, 0.6, 0.8 and 0.9% or 4, 5, 6, 8 and 9g/kg DM.

Table 4.4 Grower diet compositions (% except MJ/kg DM for energy and ppm for Zn, Cu, Mn and Fe)

	Treatment #				
Nutrient	UM_4	UM_5	UM_6	UM_8	UM_9
DM	88.94	88.96	88.98	89.01	89.06
CP	20	20	20	20	20
Energy	16	16	16	16	16
ADF	5.63	5.38	5.68	6.21	6.26
NDF	22.18	18.06	14.73	19.84	19.68
Fat	3.66	5.38	6.22	6.55	4.40
Ash	6.95	7.02	7.17	7.42	6.53
Ca	1.03	1.03	1.04	1.03	1.01
Mg	0.22	0.22	0.23	0.21	0.22
K	0.98	1.01	1.02	0.97	1.02
Na	0.27	0.29	0.31	0.26	0.34
K/Ca+Mg	0.38	0.39	0.36	0.38	0.38
P	0.77	0.76	0.82	0.71	0.76
Zn	42	49	48	48	56
Cu	8	12	15	13	9
Mn	70	94	88	63	76
Fe	316	308	326	293	334
Methionine level	0.4	0.5	0.6	0.8	0.9

: Treatment codes are explained in Chapter 3, Table 3.3 (The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed).

4.4 Male broiler chickens aged 22 to 42 days

Results of the effects of dietary methionine level on feed intake, digestibility, growth rate, feed conversion ratio (FCR), live weight, metabolisable energy intake and nitrogen retention of male Ross 308 broiler chickens aged 22 to 42 days are presented in Table 4.5. Dry matter intake of male Ross 308 broiler chickens was affected ($P<0.05$) by dietary methionine level during Week 4. Male Ross 308 broiler chickens on a diet containing 9g of methionine per kg DM had higher ($P<0.05$) DM intakes than those on diets having 4, 6 or 8g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 8g of methionine per kg DM had similar (P>0.05) dry matter intakes. Similarly, male Ross 308 broiler chickens on diets containing 5 or 9g of methionine per kg DM had the same (P>0.05) dry matter intakes.

During Week 5, male Ross 308 broiler chickens on diets containing 8 or 9g of methionine per kg DM had higher (P<0.05) DM intakes than those on diets having 5 or 6g of methionine per kg DM. Similarly, male Ross 308 broiler chickens on a diet containing 4g of methionine per kg DM had higher (P<0.05) dry matter intakes than those on diets having 5g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 5 or 6g of methionine per kg DM had similar (P>0.05) dry matter intakes. Similarly, male Ross 308 broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had the same (P>0.05) dry matter intakes. Intake of Ross 308 broiler chickens was not affected (P>0.05) by dietary methionine level during Week 6.

Dry matter digestibility of male Ross 308 broiler chickens was affected (P<0.05) by dietary methionine level during Week 6. Dietary methionine level of 6g methionine per kg DM diet had higher (P<0.05) dry matter digestibility values than those having a 4g of methionine per kg DM diet. However, male Ross 308 broiler chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had similar (P>0.05) dry matter digestibility values. Similarly, male Ross 308 broiler chickens on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same (P>0.05) dry matter digestibility values. Diets containing 6 or 9g of methionine per kg DM had higher (P<0.05) crude protein digestibility values than those on diets having 4g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5 or 8g of methionine per kg DM had similar (P>0.05) crude protein digestibility values. Similarly, male Ross 308 broiler chickens on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same (P>0.05) crude protein digestibility values.

Results of the present study indicate that dietary methionine level affected (P<0.05) NDF and ADF digestibility values. Male Ross 308 broiler chickens on diets containing 8 or 9g of methionine per kg DM had higher (P<0.05) NDF digestibility values than those having a 6g of methionine per kg DM diet. However, male Ross

308 broiler chickens on diets containing 4, 5 or 6g of methionine per kg DM had similar (P>0.05) NDF digestibility values. Similarly, male Ross 308 broiler chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had the same (P>0.05) NDF digestibility values. Male Ross 308 broiler chickens on a diet containing 9g of methionine per kg DM had higher (P<0.05) ADF digestibility values than those having 8g of methionine per kg DM diet. However, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 8g of methionine per kg DM had similar (P>0.05) ADF digestibility values. Similarly, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 9g of methionine per kg DM had the same (P>0.05) ADF digestibility values.

Fat digestibility was affected (P<0.05) by dietary methionine level (Table 4.5). Male Ross 308 broiler chickens on a diet containing 6g of methionine per kg DM had higher (P<0.05) fat digestibility values than those having diets containing 4 or 9g of methionine per kg DM diet. Similarly, male Ross 308 broiler chickens on diets containing 5, 8 or 9g of methionine per kg DM had higher (P<0.05) fat digestibility values than those having a 4g of methionine per kg DM diet. However, male Ross 308 broiler chickens on diets containing 5, 6 or 8g of methionine per kg DM had similar (P>0.05) fat digestibility values. Similarly, male Ross 308 broiler chickens on diets containing 5, 8 or 9g of methionine per kg DM had the same (P>0.05) fat digestibility values.

Male Ross 308 broiler chickens on a diet containing 6g of methionine per kg DM had higher (P<0.05) ash digestibility values than those having 5g of methionine per kg DM diet. However, male Ross 308 broiler chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had similar (P>0.05) ash digestibility values. Similarly, male Ross 308 broiler chickens on diets containing 4, 6, 8 or 9g of methionine per kg DM had the same (P>0.05) ash digestibility values.

Dietary methionine level had no effect (P>0.05) on growth rate and FCR of male Ross 308 broiler chickens aged 22 to 42 days (Table 4.5). Similarly, dietary methionine level had no effect (P>0.05) on live weight of chickens aged 28 days. However, dietary methionine level had an effect (P<0.05) on live weight of the chickens aged 35 days. Male Ross 308 broiler chickens on a diet containing 9g of methionine per kg DM had higher (P<0.05) live weights than those having diets containing 4 or 5g of methionine per kg DM. Similarly, male Ross 308 broiler chickens on diets containing 6 or 8g of methionine per kg DM had higher ($P<0.05$) live weights than those on a diet having 5g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4 or 5g of methionine per kg DM had similar ($P>0.05$) live weights. Similarly, male Ross 308 broiler chickens on diets containing 4, 6, 8 or 9g of methionine per kg DM had the same ($P>0.05$) live weights. Male Ross 308 broiler chickens aged 42 days and on a diet containing 9g of methionine per kg DM had heavier ($P<0.05$) live weights than those having a 4g of methionine per kg DM diet. However, male Ross 308 broiler chickens aged 42 days and on diets containing 4, 5, 6 or 8g of methionine per kg DM had similar ($P>0.05$) live weights. Similarly, male Ross 308 broiler chickens aged 42 days and on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same ($P>0.05$) live weights.

Metabolisable energy intake of male Ross 308 broiler chickens aged 22 to 42 days were not affected ($P>0.05$) by dietary methionine level (Table 4.5). However, dietary methionine level affected ($P<0.05$) N-retention of male Ross 308 broiler chickens aged 22 to 42 days. Male Ross 308 broiler chickens on a diet containing 6g of methionine per kg DM had higher ($P<0.05$) N-retention values than those having diets containing 5g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had similar ($P>0.05$) N-retention values. Similarly, male Ross 308 broiler chickens on diets containing 4, 6, 8 or 9g of methionine per kg DM had the same ($P>0.05$) N-retention values.

Table 4.5 Effect of dietary methionine level on diet DM intake, growth rate, live weight, digestibility, ME intake, nitrogen retention and feed conversion ratio of male Ross 308 broiler chickens aged 22 to 42 days*

	Treatment #				
Variable	M_4	M_5	M_6	M_8	M_9
DM intake (g/chicken/day)					
Week 4	97^{b}±4.0	99^{ab}±2.5	97^{b}±1.2	96^{b}±1.8	106^{a}±4.0
Week 5	143^{ab}±1.5	135^{c}±3.0	138^{bc}±2.0	144^{a}±0.4	144^{a}±14
Week 6	173^{a}±6.8	178^{a}±12.0	173^{a}±7.7	181^{a}±6.3	181^{a}±8.9
Digestibility (%), Week 6					
DM	89.2^{b}±1.71	90.7^{ab}±0.58	91.6^{a}±0.52	91.0^{ab}±1.18	90.7^{ab}±0.90
CP	39.8^{b}±7.52	43.7^{ab}±1.96	56.0^{a}±6.00	52.7^{ab}±2.00	54.8^{a}±2.55
NDF	34.0^{ab}±9.46	29.7^{ab}±3.52	25.5^{b}±5.72	38.7^{a}±2.82	37.9^{a}±5.98
ADF	20.7^{ab}±0.55	20.3^{ab}±2.02	20.1^{ab}±2.17	17.8^{b}±0.80	23.7^{a}±2.39
Fat	64.5^{c}±4.71	80.4^{ab}±1.20	85.0^{a}±2.01	82.5^{ab}±0.76	75.8^{b}±1.11
Ash	20.9^{ab}±5.70	19.4^{b}±2.16	30.5^{a}±8.17	28.2^{ab}±2.04	24.2^{ab}±6.00
Growth rate (g/chicken/day)					
Week 4	37^{a}±7.0	35^{a}±1.0	38^{a}±4.5	40^{a}±5.0	42^{a}±1.8
Week 5	111^{a}±1.7	103^{a}±3.2	109^{a}±0.8	111^{a}±5.9	114^{a}±2.7
Week 6	77^{a}±2.3	94^{a}±13.9	88^{a}±4.2	96^{a}±4.4	95^{a}±22.6
FCR (g feed intake/g weight gain)					
Week 4	2.8^{a}±0.54	2.9^{a}±0.12	2.6^{a}±0.25	2.5^{a}±0.34	2.5^{a}±0.18
Week 5	1.3^{a}±0.01	1.3^{a}±0.01	1.3^{a}±0.02	1.3^{a}±0.07	1.3^{a}±0.03
Week 6	2.2^{a}±0.04	1.9^{a}±0.18	2.0^{a}±0.02	1.9^{a}±0.15	2.1^{a}±0.32
Live weight (g/chicken)					
Day 28	910^{a}±30.7	919^{a}±1.2	946^{a}±12.9	937^{a}±27.1	963^{a}±12.2
Day 35	1686^{bc}±19.5	1643^{c}±23.5	1710^{ab}±9.0	1712^{ab}±24.3	1758^{a}±20.6
Day 42	2226^{b}±15.1	2298^{ab}±111.4	2328^{ab}±37.4	2385^{ab}±6.5	2423^{a}±146.8
ME(MJ/bird/d)	11.4^{a}±0.20	11.5^{a}±0.26	11.6^{a}±0.30	11.6^{a}±0.28	11.5^{a}±0.26
N-re (g/bird/d)	29.6^{ab}±5.85	26.0^{b}±0.25	40.9^{a}±7.24	34.0^{ab}±1.90	39.2^{ab}±2.0

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

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

: Treatment codes are explained in Chapter 3, Table 3.3 (The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed)

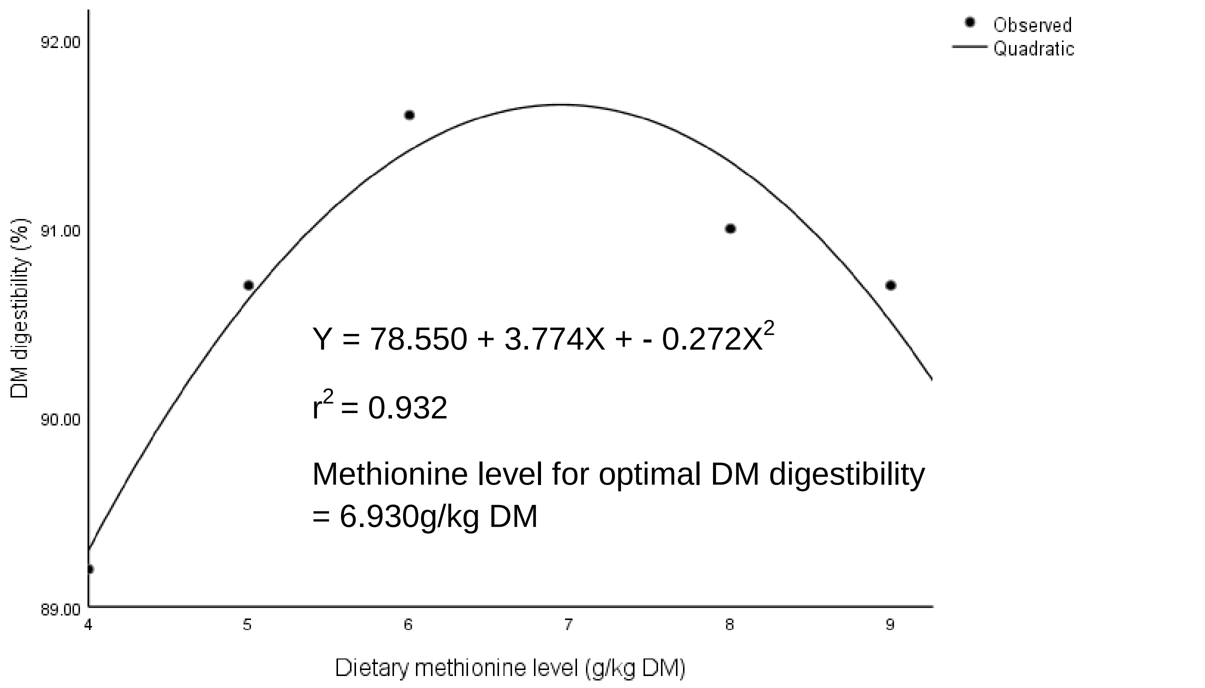

Figure 4.7 Effect of dietary methionine level on DM digestibility in male Ross 308 broiler chickens aged 22 to 42 days

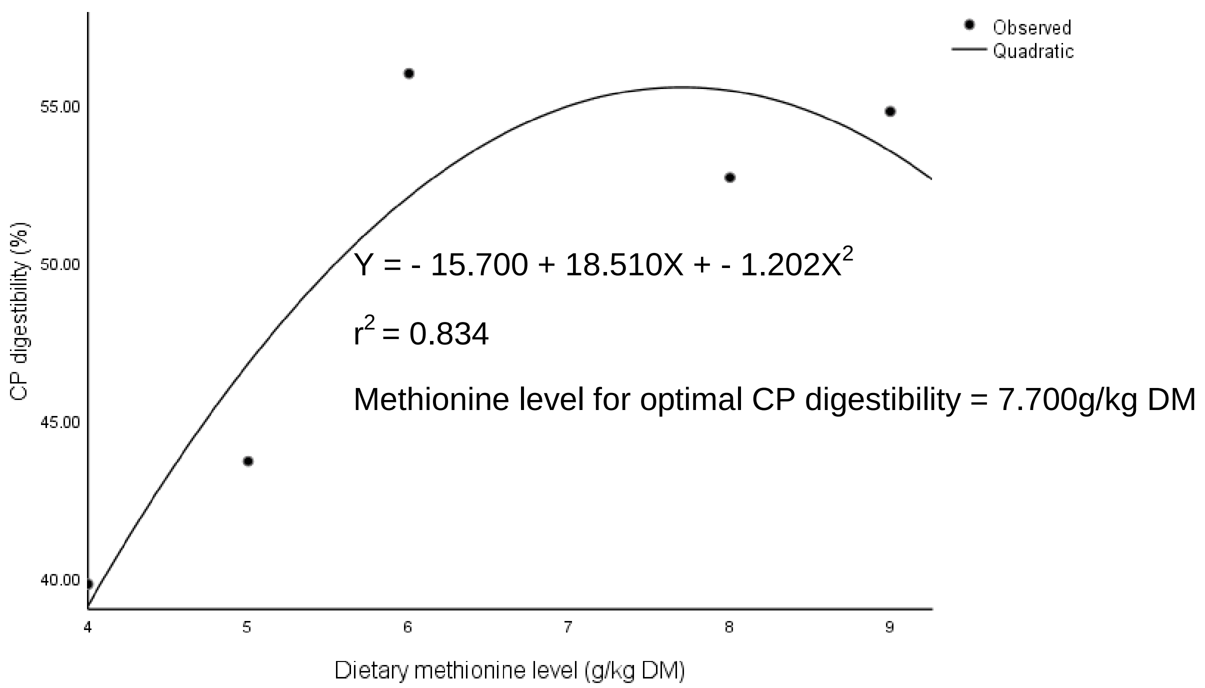

Figure 4.8 Effect of dietary methionine level on CP digestibility in male Ross 308 broiler chickens aged 22 to 42 days

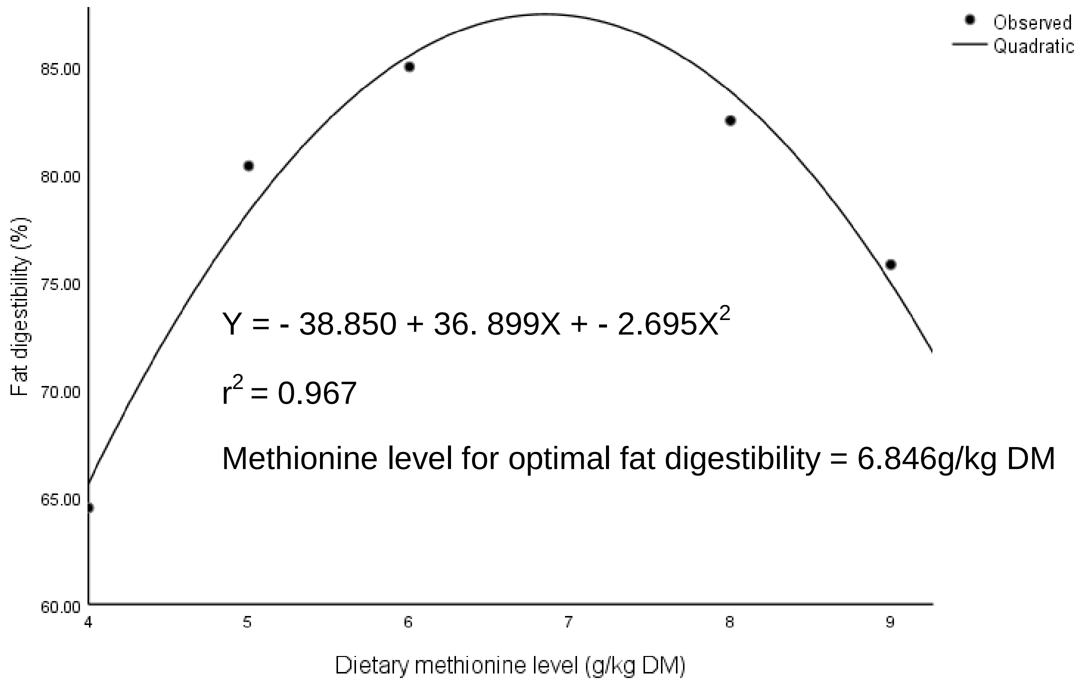

Figure 4.9 Effect of dietary methionine level on fat digestibility in male Ross 308 broiler chickens aged 42 days

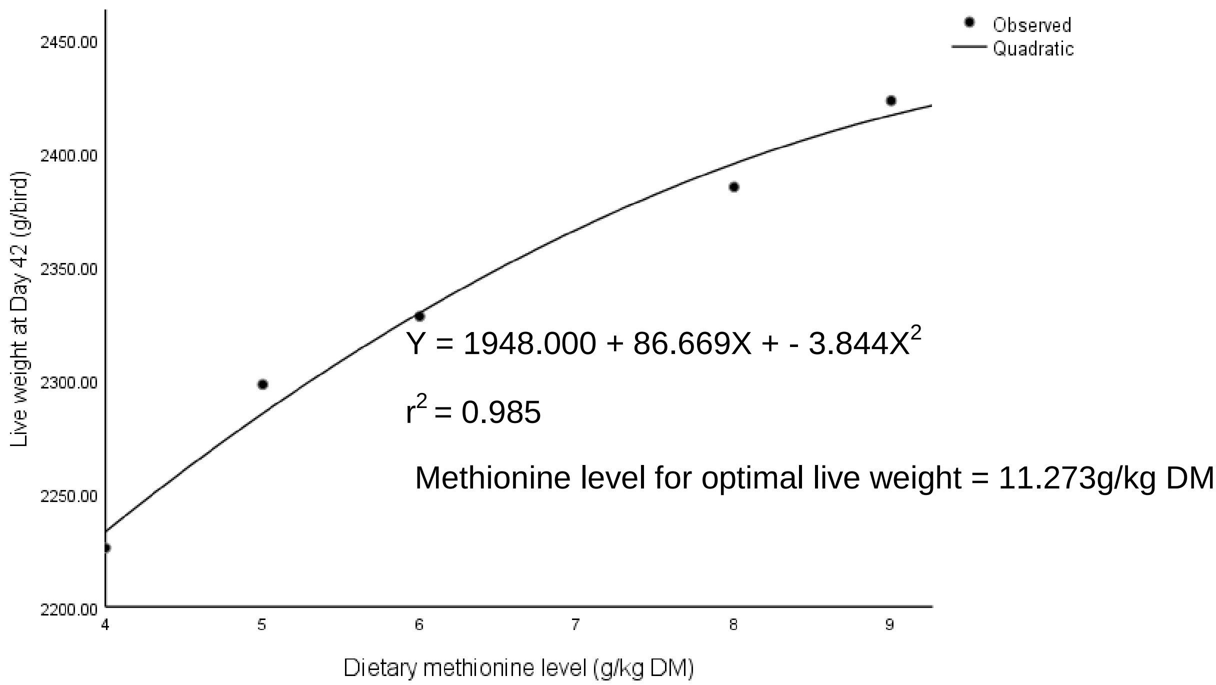

Figure 4.10 Effect of dietary methionine level on live weight of male Ross 308 broiler chickens aged 42 days

Dry matter digestibility, CP digestibility, fat digestibility and live weights of male Ross 308 broiler chickens aged 42 days were optimized at dietary methionine levels of 6.930 (r^2 = 0.932), 7.700 (r^2 = 0.834), 6.846 (r^2 = 0.967) and 11.273g (r^2 = 0.985) g/kg DM, respectively (Figures 4.7, 4.8, 4.9 and 4.10 respectively).

Results of the effects of dietary methionine level on gut organ lengths, weights and digesta pH of male Ross 308 broiler chickens aged 42 days are presented in Table 4.6. Dietary methionine level had effects on crop weights of the chickens. Male Ross 308 broiler chickens on diets containing 6g of methionine per kg DM had higher (P<0.05) crop weights than those on diets having 4g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had similar (P>0.05) crop weights. Similarly, male Ross 308 broiler chickens on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same (P>0.05) crop weights.

Dietary methionine level had no effect (P>0.05) on proventriculus, gizzard, caecum and large intestine weights of male Ross 308 broiler chickens aged 42 days (Table 4.6). However, dietary methionine level had effects (P<0.05) on small intestine weights of male Ross 308 broiler chickens. Male Ross 308 broiler chickens on diets containing 8g of methionine per kg DM had higher (P<0.05) small intestine weights than those having diets containing 6g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 9g of methionine per kg DM had similar (P>0.05) small intestine weights. Similarly, male Ross 308 broiler chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had the same (P>0.05) small intestine weights.

Male Ross 308 broiler chicken GIT lengths were affected (P<0.05) by dietary methionine level (Table 4.6). Male Ross 308 broiler chickens on diets containing 9g of methionine per kg DM had longer (P<0.05) GIT lengths than those having diets containing 4g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 8g of methionine per kg DM had similar (P>0.05) GIT lengths. Similarly, male Ross 308 broiler chickens on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same (P>0.05) GIT lengths. Dietary methionine level of male Ross 308 broiler chickens had no effect (P>0.05) on small intestine, caecum and large intestine lengths.

Results of the present study indicate that dietary methionine level had no effect (P>0.05) on crop, gizzard, caecum and large intestine digesta pH values of male broiler chickens aged 42 days (Table 4.6). However, dietary methionine level had effects (P<0.05) on proventriculus digesta pH values. Male Ross 308 broiler chickens on a diet containing 9g of methionine per kg DM had higher (P<0.05) proventriculus digesta pH values than those having diets containing 6g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 8g of methionine per kg DM had similar (P>0.05) proventriculus digesta pH values. Similarly, male Ross 308 broiler chickens on diets containing 4, 5, 8 or 9g of methionine per kg DM had the same (P>0.05) proventriculus digesta pH values.

Small intestine digesta pH of male Ross 308 broiler chickens was affected (P<0.05) by dietary methionine level (Table 4.6). Male Ross 308 broiler chickens on diets containing 4g of methionine per kg DM had higher (P<0.05) small intestine digesta pH values than those having diets containing 5 or 6g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had similar (P>0.05) small intestine digesta pH values. Similarly, male Ross 308 broiler chickens on diets containing 5, 6, 8 or 9g of methionine per kg DM had the same (P>0.05) small intestine digesta pH values.

Table 4.6 Effect of dietary methionine level on gut organ digesta pH, length and weight of male Ross 308 broiler chickens aged 42 days*

	Treatment #				
Variable	Met_4	Met_5	Met_6	Met_8	Met_9
Gut organ weight (g)					
Crop	8^{b}±0.7	9^{ab}±0.9	12^{a}±1.0	10^{ab}±2.1	8^{ab}±1.3
Proventriculus	8^{a}±0.7	10^{a}±1.0	9^{a}±0.1	10^{a}±0.7	9^{a}±1.8
Gizzard	28^{a}±2.7	31^{a}±3.3	32^{a}±4.9	37^{a}±5.7	29^{a}±3.6
Small intestine	99^{ab}±2.4	110^{ab}±5.0	93^{b}±4.0	116^{a}±8.5	106^{ab}±6.6
Caecum	5^{a}±1.5	8^{a}±0.9	5^{a}±0.4	7^{a}±1.7	6^{a}±1.2
Large intestine	5^{a}±1.5	7^{a}±1.1	5^{a}±0.4	6^{a}±0.9	5^{a}±0.9
Gut organ length (cm)					
GIT	212^{b}±3.1	217^{ab}±9.4	224^{ab}±8.0	224^{ab}±2.5	225^{a}±1.9
Small intestine	182^{a}±5.9	186^{a}±6.7	177^{a}±11.4	191^{a}±1.3	189^{a}±3.9
Caecum	19^{a}±0.3	20^{a}±0.7	20^{a}±0.9	20^{a}±0.5	20^{a}±0.7
Large intestine	12^{a}±0.6	12^{a}±0.5	11^{a}±0.6	12^{a}±0.5	13^{a}±0.7
Gut organ digesta pH					
Crop	4.4^{a}±0.16	4.2^{a}±0.03	4.3^{a}±0.21	4.6^{a}±0.68	4.2^{a}±0.25
Proventriculus	4.2^{ab}±0.48	4.0^{ab}±0.33	4.0^{b}±0.8	3.9^{ab}±0.24	3.5^{a}±0.30
Gizzard	2.6^{a}±0.25	2.5^{a}±0.13	2.7^{a}±0.23	2.1^{a}±0.15	2.3^{a}±0.28
Small intestine	5.7^{a}±0.21	5.0^{b}±0.29	5.0^{b}±0.27	5.6^{ab}±0.18	5.4^{ab}±0.13
Caeca	6.2^{a}±0.8	6.1^{a}±0.13	5.8^{a}±0.11	6.4^{a}±0.06	5.7^{a}±0.18
Large intestine	5.6^{a}±0.36	6^{a}±0.2	5.3^{a}±0.15	5.0^{a}±0.15	5.8^{a}±0.31

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

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

: Treatment codes are explained in Chapter 3, Table 3.3 (The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed)

Crop weight and gastrointestinal tract lengths of male Ross 308 broiler chickens aged 42 days were optimized at dietary methionine levels of 6.558 (r^2 = 0.840) and 7.851(r^2 = 0.950) g/kg DM, respectively (Figures 4.11 and 4.12, respectively). There was a negative relationship (r = 0.903) between dietary methionine level and proventriculus digesta pH (Figure 4.13).

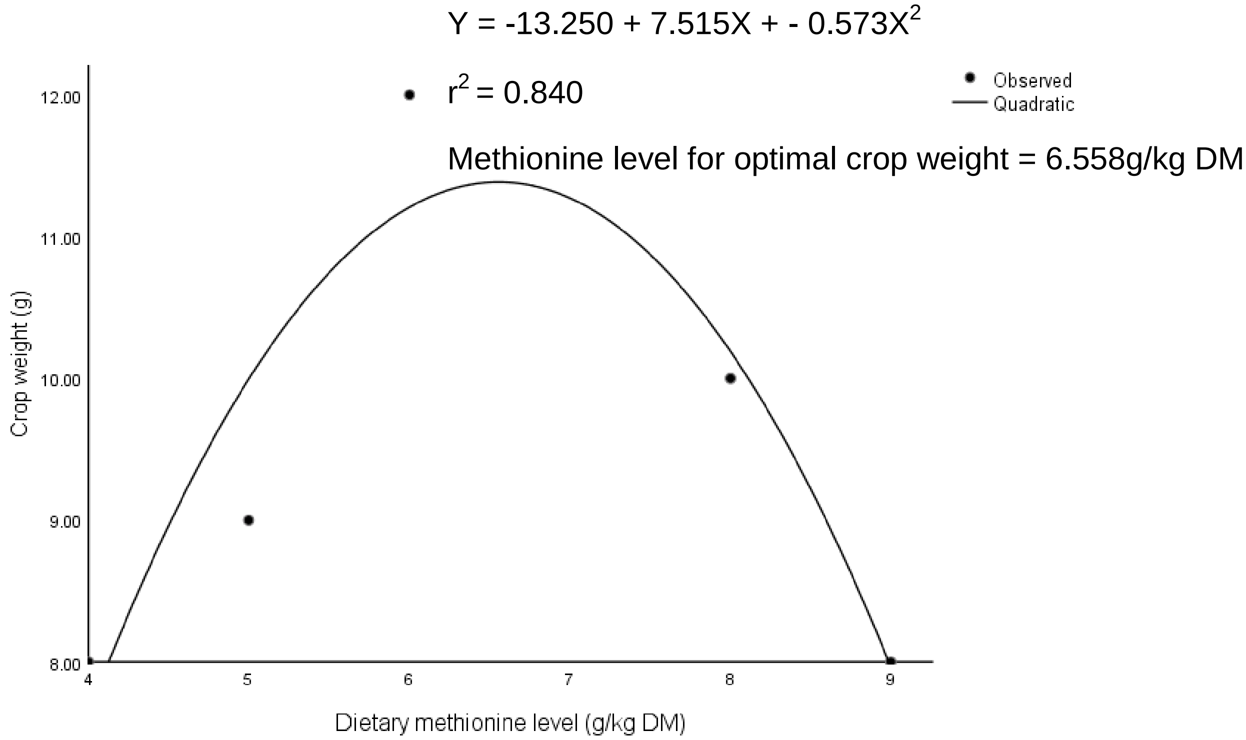

Figure 4.11 Effect of dietary methionine level on crop weight of male Ross 308 broiler chickens aged 42 days

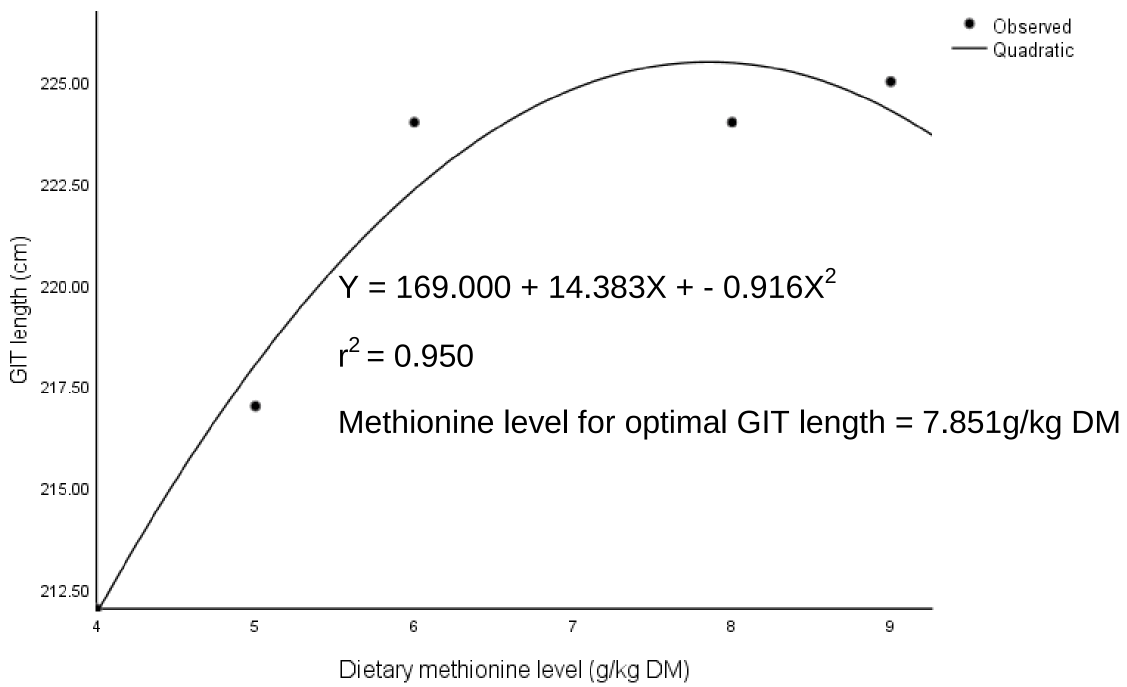

Figure 4.12 Effect of dietary methionine level on GIT length of male Ross 308 broiler chickens aged 42 days

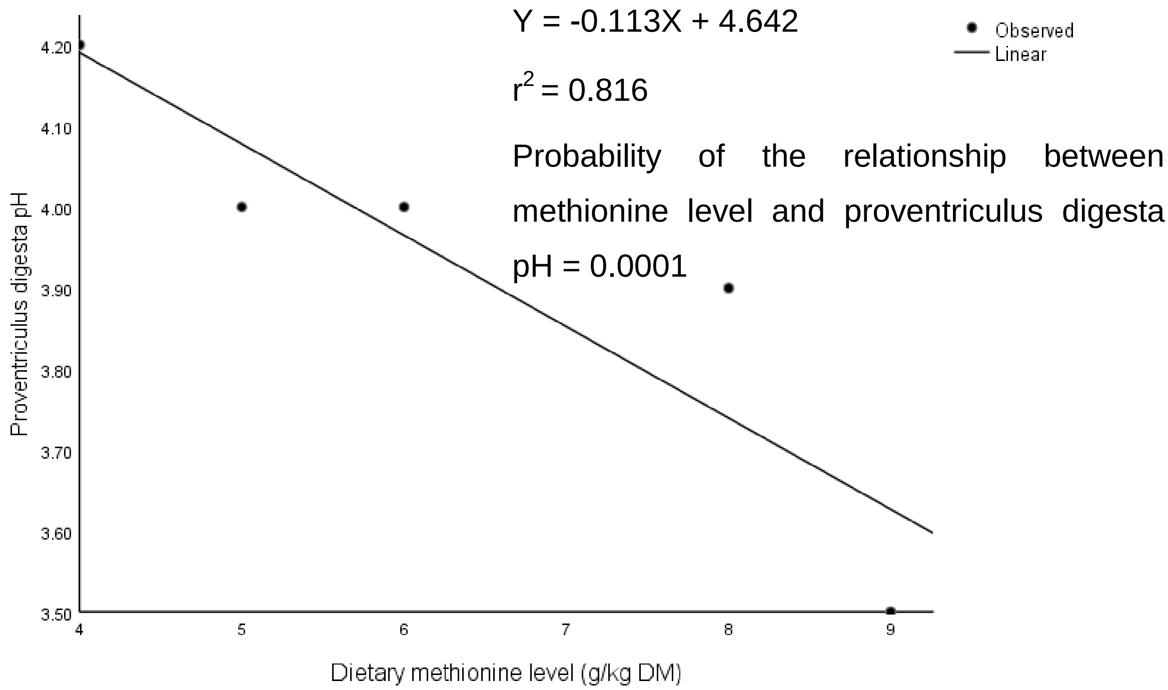

Figure 4.13 Relationship between dietary methionine level and proventriculus digesta pH of male Ross 308 broiler chickens aged 42 days

Results of the effects of dietary methionine level on carcass, breast, drumstick and thigh weights of male Ross 308 broiler chickens aged 42 days are presented in Table 4.7. Dietary methionine level affected (P<0.05) carcass weights of male Ross 308 broiler chickens. Male Ross 308 broiler chickens on diets containing 8g of methionine per kg DM had higher (P<0.05) carcass weights than those having diets containing 4 or 5g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4 or 5g of methionine per kg DM had similar (P>0.05) carcass weights. Similarly, male Ross 308 broiler chickens on diets containing 6, 8 or 9g of methionine per kg DM had the same (P>0.05) carcass weights.

Breast weight of male Ross 308 broiler chickens was affected (P<0.05) by dietary methionine level (Table 4.7). Male Ross 308 broiler chickens on diets containing 8g of methionine per kg DM had higher (P<0.05) breast weights than those having diets containing 5 or 6g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 9g of methionine per kg had similar (P>0.05) breast weights. Similarly, male Ross 308 broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had the same (P>0.05) breast weights. Male Ross 308 broiler chickens on diets containing 8 or 9g of methionine per kg DM had higher (P<0.05) drumstick weights than those having diets containing 5 or 6g of methionine per kg DM (Table 4.7). However, male Ross 308 broiler chickens on diets containing 4, 8 or 9g of methionine per kg had similar (P>0.05) drumstick weights. Similarly, male Ross 308 broiler chickens on diets containing 5 or 6g of methionine per kg DM had the same (P>0.05) drumstick weights.

Results of the present study indicate that dietary methionine level had effects (P<0.05) on thigh weights of male Ross 308 broiler chickens aged 42 days. Male Ross 308 broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had heavier (P<0.05) thigh weights than those having diets containing 5 or 6g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 8 or 9g of methionine per kg DM had similar (P>0.05) thigh weights. Similarly, male Ross 308 broiler chickens on diets containing 5 or 6g of methionine per kg DM had the same (P>0.05) thigh weights.

Table 4.7 Effect of dietary methionine level on carcass organ weights (g) of male Ross 308 broiler chickens*

	Treatment #				
Variable	Met_4	Met_5	Met_6	Met_8	Met_9
Carcass	1533^{bc}±63.0	1471^{c}±45.8	1635^{ab}±55.2	1708^{a}±12.5	1649^{ab}±45.2
Breast	553^{ab}±25.3	538^{b}±32.9	524^{b}±39.0	629^{a}±25.1	601^{ab}±4.9
Drumstick	195^{ab}±7.9	178^{c}±3.5	191^{bc}±17.3	207^{a}±10.8	209^{a}±8.6
Thigh	246^{a}±11.7	225^{b}±4.4	218^{b}±20.3	255^{a}±4.3	249^{a}±11.1

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

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

: Treatment codes are explained in Chapter 3, Table 3.3 (The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed)

There was a positive relationship (r = 0.758) between dietary methionine level and breast weight of the chickens (Figure 4.14).

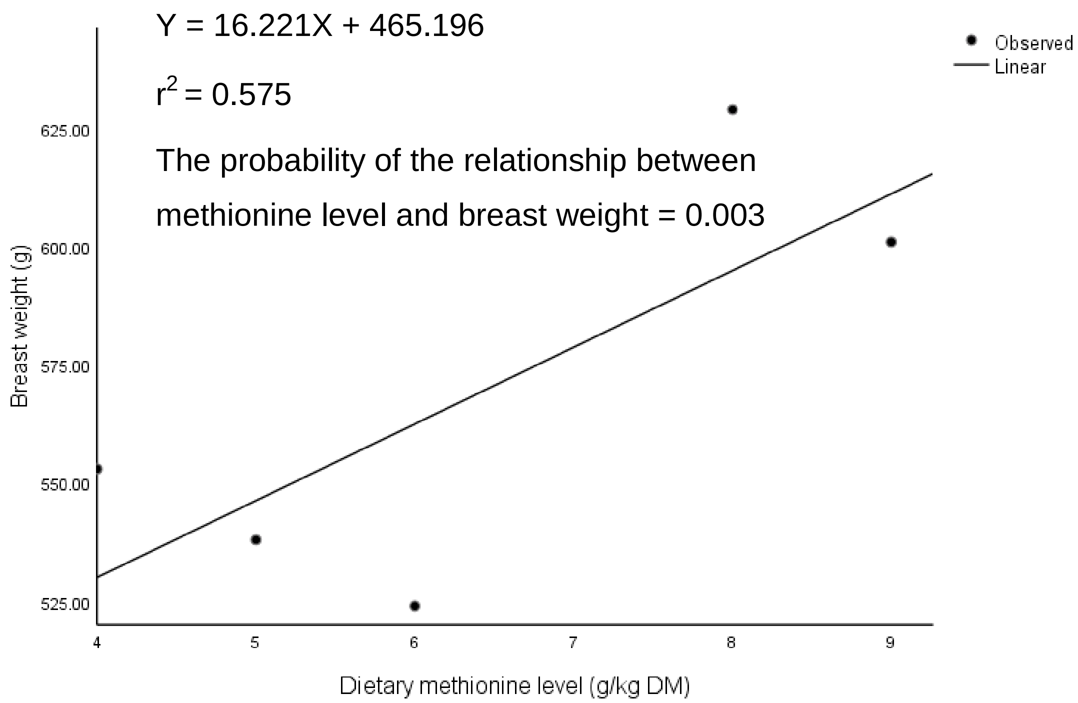

Figure 4.14 Relationship between dietary methionine level and breast weight of male Ross 308 broiler chickens aged 42 days

Results of the effects of dietary methionine level on male Ross 308 broiler chicken meat tenderness, juiciness, flavour and shear force are presented in Table 4.8. Dietary methionine level had an effect (P<0.05) on male Ross 308 broiler chicken meat tenderness. Male Ross 308 broiler chickens on diets containing 9g of methionine per kg DM had higher (P<0.05) meat tenderness values than those having diets containing 4 or 5g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing 4, 5, 6 or 8g of methionine per kg DM had similar (P>0.05) meat tenderness values. Similarly, male Ross 308 broiler chickens on diets containing 6, 8 or 9g of methionine per kg DM had the same (P>0.05) meat tenderness values.

Meat Juiciness was affected (P<0.05) by dietary methionine level (Table 4.8). Male Ross 308 broiler chickens on diets containing 6 or 9g of methionine per kg DM had higher (P<0.05) meat juiciness values than those having diets containing 4 or 5g of methionine per kg DM. However, male Ross 308 broiler chickens on diets containing

4, 5 or 8g of methionine per kg DM had similar (P>0.05) meat juiciness values. Similarly, male Ross 308 broiler chickens on diets containing 6, 8 or 9g of methionine per kg DM had the same (P>0.05) meat juiciness values. Dietary methionine level had no effect (P>0.05) on meat flavour and shear force values of male Ross 308 broiler chickens aged 42 days.

Table 4.8 Effect of dietary methionine level on meat tenderness, juiciness, flavour and shear force of male Ross 308 broiler chickens*

	Treatment #				
Variable	Met_4	Met_5	Met_6	Met_8	Met_9
Tenderness	3.5^{b}±0.15	3.5^{b}±0.37	3.8^{ab}±0.10	3.8^{ab}±0.06	3.9^{a}±0.06
Juiciness	3.3^{b}±0.13	3.3^{b}±0.26	3.7^{a}±0.24	3.6^{ab}±0.15	3.8^{a}±0.15
Flavour	3.2^{a}±0.15	3.5^{a}±0.28	3.5^{a}±0.24	3.6^{a}±0.28	3.4^{a}±0.12
Shear force	11.6^{a}±2.52	9.7^{a}±4.32	11.1^{a}±2.02	10.4^{a}±0.80	8.2^{a}±1.30

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

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

: Treatment codes are explained in Chapter 3, Table 3.3 ((The treatments were dietary methionine levels of 4, 5, 6, 8 or 9g/kg DM feed)

Broiler chicken meat tenderness and juiciness were optimized at dietary methionine levels of 10.087 (r^2 = 0.841) and 13.321 (r^2 = 0.745) g/kg DM, respectively (Figures 4.15 and 4.16, respectively).

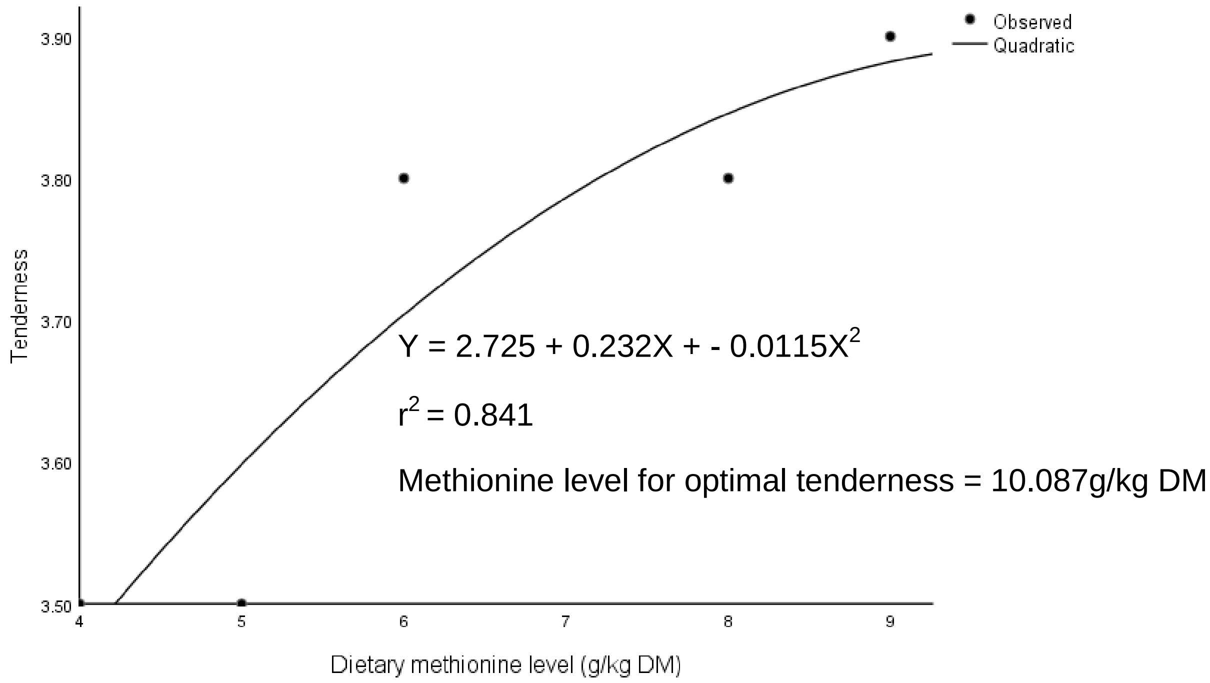

Figure 4.15 Effect of dietary methionine level on meat tenderness of male Ross 308 broiler chickens aged 42 days

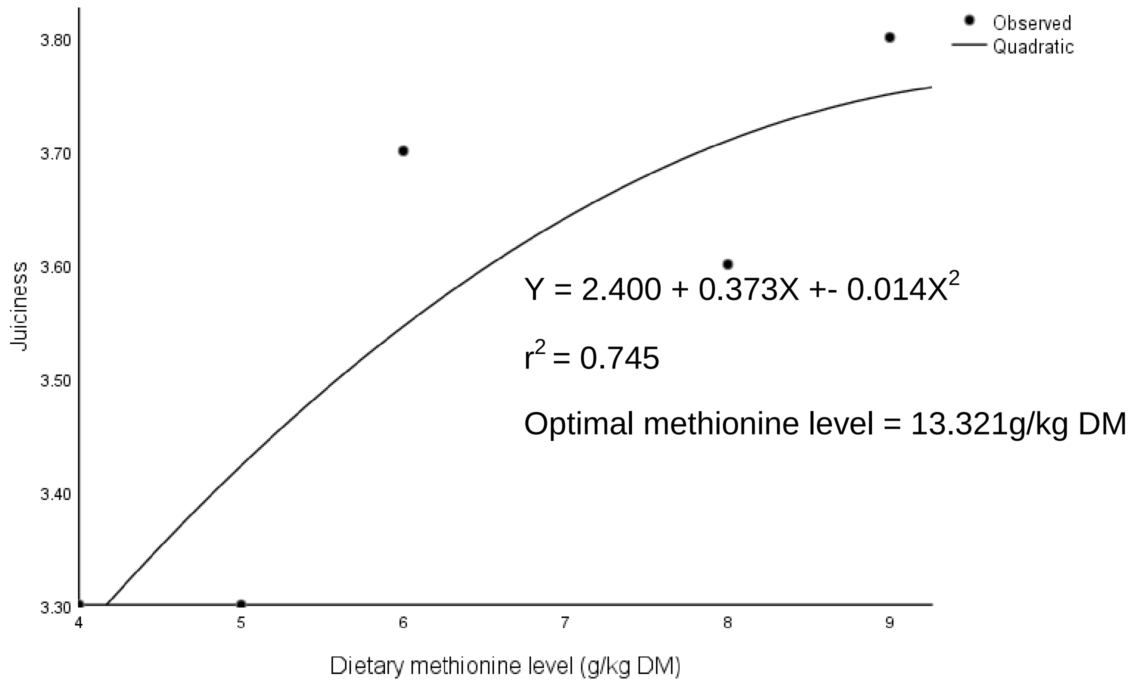

Figure 4.16 Effect of dietary methionine level on meat juiciness of male Ross 308 broiler chickens aged 42 days.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

5.1.1 Unsexed broiler chickens aged one to 21 days

The diets contained 22% CP and 16.2MJ of energy/kg DM. This met the recommended requirements for the broiler chickens aged one to 21 days (NRC, 1994). The diets contained similar levels of all the nutrients except dietary methionine level which ranged from 4 to 9g/kg DM.

The present results indicate that dietary methionine level did not have effect on DM intake of unsexed Ross 308 broiler chickens aged one to 21 days. This is similar to the findings of Yalcin *et al*. (1999) who observed no significant differences in feed intake across the treatments, but contrary to the findings of Halder and Roy (2007) who reported increased intake for the chickens supplemented with DL-methionine. Results of the current study are contrary to the findings of other studies (Bunchasak and Keawarun, 2006; Zhan *et al*., 2006), who observed that adding supplemental methionine to a diet increased feed consumption of the chickens. Esteve-Garcia and Llaurado (1997) reported that supplemental methionine reduced feed intake. Opoola *et al*. (2016) found that a diet with 0.65 % methionine had the highest feed intake. The present results are contrary to those of Elwinger and Tausen (2009) who reported significantly high feed intake with increasing methionine levels in the diet of broiler chickens. The study by Charles *et al*. (2017) observed that the addition of 0.25% and 0.14% methionine into diets at the starter phase increased feed intake by 26.56 and 20.28%, respectively.

Dietary methionine level did not have effect on CP, ADF, NDF and fat digestibilities by Ross 308 broiler chickens. However, dietary methionine level improved DM and ash digestibilities. No studies on the effect of dietary methionine level on diet digestibility of unsexed broiler chickens were found.

Results of the present study indicate that feed conversion ratio (FCR) of unsexed Ross 308 broiler chickens aged one to 21 days was not affected by the increase in dietary methionine level. This is similar to the findings of Golshahi *et al*. (2013) who reported no significant differences in FCR with increase dietary methionine level. Similarly, Mohsen *et al*. (2012) did not find any effect of dietary methionine level on

FCR of unsexed Ross 308 broiler chickens aged one to 21 days. Café and Waldroup (2006) found that methionine level had no significant effect on feed conversion ratio of chickens aged one to 16 days. However, the present results are contrary to those of Bunchasak and Keawarum (2006) and Zhan *et al*. (2006) who reported improved FCR in response to increase in dietary methionine level. Similarly, Hickling *et al*. (1990) observed increases in feed conversion ratio with increasing levels of dietary methionine.

The present study indicates that dietary methionine level had no effect on growth rate of unsexed Ross 308 broiler chickens aged one to 21 days. This was possibly due to insignificant differences in feed intake, FCR, metabolisable energy intake and nitrogen retention. The results are contrary to those of Yaqoob and Mubarak, (2018) who observed that increase in dietary methionine level improved FCR and growth rates of broiler chickens aged one to 21 days. This is similar to the findings of other authors who have reported that dietary methionine level above the recommended requirements (0.50%) of broiler chickens improved their performance in terms of body weight gain (Drażbo *et al*., 2015; Adbalqdadir and Arabi, 2014; Zhai *et al*., 2016).

Results of the present study indicate that dietary methionine level did not improve live weight of unsexed Ross 308 broiler chickens aged 7 or 14 days. In fact, higher dietary methionine levels resulted in lower live weights of the chickens. Dietary methionine level did not have any effect on live weight of the chickens aged 21 days. This is similar to the findings of Kidd *et al*. (1997) who observed no improvement in live weights of broiler chickens aged 7 or 14 days and fed diets supplemented with methionine. However, the present results are contrary to those of Opoola *et al*. (2016) who found that broiler chickens fed a dietary methionine level of 0.60% had the best result in terms of final weight and weight gain during the starter period.

Dietary methionine level did not have any effect on metabolisable energy intake of Ross 308 broiler chickens aged one to 21 days. Similarly, dietary methionine level did not have any effect on nitrogen retention of unsexed Ross 308 broiler chickens aged one to 21 days. Kalinowski *et al*. (2003) found that nitrogen retention in broiler chickens aged one to 21 days was optimized at 0.46% methionine level. Information on the effect of dietary methionine on nitrogen retention and metabolisable energy intake of unsexed Ross 308 broiler chickens is limited. Thus, there is a need for further studies.

The results of the present study indicate that dietary methionine level did not have any effect on crop, gizzard and small intestine weights of unsexed Ross 308 broiler chickens aged 21 days. This is similar to the findings of Saki *et al*. (2007) who reported that increasing dietary methionine level did not affect crop, gizzard and small intestine weights of unsexed broiler chickens aged 21 days. However, dietary methionine level improved proventriculus and large intestine weights of unsexed broiler chickens aged 21 days. This is contrary to the findings of Saki *et al*. (2007) who observed that dietary methionine level did not affect proventriculus and large intestine weights. Saki *et al*. (2007) observed that any increase above the NRC (1994) recommendation of 0.55% methionine did not have any effect on gut organ weights. In the present study, dietary methionine level did not improve caecum weight of unsexed Ross 308 broiler chickens. In fact, higher dietary methionine levels resulted in lower caecum weights of the chickens. The results are similar to those of Saki *et al*. (2007) who found that increasing dietary methionine level did not affect caecum weights of broiler chickens aged 21 days.

Results of the present study indicate that increasing dietary methionine level improved gastrointestinal tract and small intestine lengths. However, dietary methionine level did not improve caecum and large intestine lengths. In fact, higher dietary methionine levels resulted in lower caecum and large intestine lengths. No studies on the effect of dietary methionine level on gut organ lengths were found.

Dietary methionine level did not affect crop, proventriculus and gizzard digesta pH of unsexed Ross 308 broiler chickens aged 21 days. However, increasing dietary methionine level increased small and large intestine digesta pH values. Dietary methionine level did not improve ceacum digesta pH values. No studies on the effect of dietary methionine level on gut organ digesta pH values of unsexed broiler chickens were found.

5.1.2 Male broiler chickens aged 22 to 42 days

The diets contained 20% CP and 16.0MJ of energy/kg DM. This met the recommended requirements for the broiler chickens aged 22 to 42 days (NRC, 1994). The diets contained similar levels of all the nutrients except dietary methionine level. The diets contained dietary methionine levels which ranged from 4 to 9g/kg DM.

The present results indicate that increasing dietary methionine level improved feed intake of male Ross 308 broiler chickens aged 4 and 5 weeks. This is similar to the findings of Ahmed and Abbas (2011) who observed that dietary methionine levels of 110 and 130% above those of NRC (1994) levels improved feed intake of broiler chickens. The present results, also, agree with the findings of Pillai *et al*. (2006) who reported that feed intake was significantly maximized with the addition of methionine. On the other hand, results obtained for feed intake in the present study are in disagreement with those of Saki *et al*. (2007) who did not find significant differences with increase in dietary methionine levels. Dietary methionine level in the present study did not have effect on feed intake of male Ross 308 broiler chickens aged 6 weeks. This is similar to the findings of Mohammadi *et al*. (2009) who reported that increasing levels of dietary methionine did not affect feed intake. However, the present results are contrary to those of Ojano-Dirain and Waldroup (2002) who found a decline in feed intake with increasing dietary methionine levels.

Results of the present study indicate that increasing dietary methionine level affected DM, CP, ADF, NDF, fat and ash digestibilities of male Ross 308 broiler chickens aged 35 to 42 days. Dry matter, CP and fat digestibilities were optimized at different dietary methionine levels of 6.930, 7.700 and 6.846 g/kg DM, respectively. These values are above those recommended by NRC (1994). No other studies on the effect of dietary methionine level on diet digestibility of male Ross 308 broiler chickens were found.

Dietary methionine level did not have any effect on growth rate of male Ross 308 broiler chickens aged 22 to 42 days. These results are contrary to those of Chen *et al*. (2013) who reported that a higher concentration of dietary methionine level of 5.9

g/kg DM improved growth performance of broiler chickens aged 22 to 42 days. Similarly, Osti and Pandey (2004), Pillai *et al*. (2006) and Ahmed and Abbas (2011) observed higher broiler chicken growth rates with increased dietary methionine levels.

The present results indicate that dietary methionine level did not have effect on feed conversion ratio of male Ross 308 broiler chickens aged 22 to 42 days. This is similar to the findings of Meirelles *et al*. (2003) who observed no significant effects of increasing dietary methionine levels on FCR of broiler chickens aged 22 to 42 days. However, the present results are contrary to those of Pillai *et al*. (2006) and Ahmed and Abbas (2011) who observed that the addition of dietary methionine above the recommended (NRC, 1994) requirements of broiler chickens improved their feed conversion ratio. Drazbo *et al*. (2015) observed a 10% improvement in FCR with increased dietary methionine levels. Café and Waldroup (2006) found that increasing dietary methionine levels by 115% of the NRC (1994) recommended level improved feed conversion ratio of broiler chickens aged 22 to 42 days. However, the authors observed that increasing the dietary methionine level to 130% of that of NRC (1994) recommended level did not have any effect on FCR of broiler chickens aged 22 to 42 days.

Live weights of male Ross 308 broiler chickens aged 28 days were not affected by dietary methionine level. This is similar to the findings of Hickling *et al*. (1990), Moran (1994), Schutte and Pack (1995) and Kalinowski *et al*. (2003a,b) who reported that methionine levels above NRC (1994) recommendations did not affect body weight of broiler chickens. The present results are also similar to those of Osti and Pandey (2004) who did not observe significant differences in live weights of broiler chickens with increased dietary methionine levels. However, in the present study dietary methionine level affected live weights of male Ross 308 broiler chickens aged 35 and 42 days. Dietary methionine level of 11.273 g/kg DM optimized live weights of male Ross 308 broiler chickens aged 42 days. This is similar to the findings of Café and Waldroup (2006) who found that increasing dietary methionine levels by 115% above the NRC (1994) recommendations significantly improved the live body weights of male Ross 308 broiler chickens. However, increasing dietary methionine level to 130% of the NRC (1994) recommended levels did not improve live weights of the broiler chickens. Opoola *et al*. (2016) and Chattopadhyay *et al*. (2006), also, observed similar results.

In the present study, dietary methionine level did not have effect on metabolisable energy intake of male Ross 308 broiler chickens aged 22 to 42 days. However, dietary methionine level improved nitrogen retention of male Ross 308 broiler chickens aged 22 to 42 days. The present results are similar to those of Koreleski and Świątkiewicz (2009) who observed that increasing dietary methionine level improved nitrogen retention in broiler chickens aged 22 to 42 days. Information on the effect of dietary methionine level on metabolisable energy intake and nitrogen is limited. Therefore, there is a need for further studies on these topics.

Dietary methionine level did not have any effect on proventriculus, gizzard, ceacum and large intestine weights of male Ross 308 broiler chickens aged 42 days. This is similar to the findings of Saki *et al*. (2007) who observed that increasing dietary methionine level above those recommended by NRC (1994) did not affect digestive organ weights. However, the results of the present study indicate that dietary methionine level affected crop and small intestine weights of male Ross 308 broiler chickens aged 42 days. Dietary methionine level of 6.55 g/kg DM optimized crop weights of male broiler chickens aged 42 days. The present results are contrary to the findings of Saki *et al*. (2007) that digestive organs weights of broiler chickens aged 42 days were not affected by dietary methionine level above those recommended by NRC (1994).

Dietary methionine level did not have effect on caecum, small and large intestine lengths. However, results of the present study indicate that increasing dietary methionine level improved gastrointestinal tract lengths of male Ross 308 broiler chickens aged 42 days. Male broiler chickens on diets having 9g of methionine per kg DM had the highest gastrointestinal tract length. No studies on the effect of dietary methionine level on gut organ lengths were found.

Dietary methionine level in the present study did not have effect on crop, gizzard, ceacum and large intestine digesta pH values of male Ross 308 broiler chickens aged 42 days. However, dietary methionine level affected proventriculus and small intestine digesta pH values of male Ross 308 broiler chickens aged 42 days. No particular trend was observed on the effect of dietary methionine level on crop, gizzard, caecum and large intestine digesta pH values.

Results of the present study indicate that dietary methionine level affected carcass organ weights of male Ross 308 broiler chickens aged 42 days. Increasing dietary methionine level increased chicken breast meat weight. However, there was no clear trend for the other carcass organs. Drazbo *et al*. (2015) reported improved carcass and breast meat weights of male broiler chickens on higher than NRC (1994) dietary methionine levels. Similarly, Nasr (2011) observed that broiler chickens on diets with higher methionine levels had higher carcass, breast, thigh and drumstick weights. However, Jiao *et al*. (2010) observed no effect of dietary methionine level on carcass characteristics.

Dietary methionine level did not have any effect on male Ross 308 broiler chicken meat flavour and shear force values. This is contrary to the findings of Jiao *et al*. (2010) that higher levels of dietary methionine reduced shear force values of broiler chicken meat. Similarly, Zonenberg and Drazbo (2018) observed that dietary methionine level of 0.08% had the best meat flavour of male broiler chickens aged 42 days. Results of the present study indicate that dietary methionine level had effect on male broiler chicken meat juiciness and tenderness, respectively. Dietary methionine levels of 10.087 and 13.321 g/kg DM optimized broiler chicken meat tenderness and juiciness. Zonenberg and Drazbo (2018) reported better meat tenderness and juiciness for male broiler chickens on diets having 0.08 and 0.24% methionine, respectively. However, Drazbo *et al*. (2015) reported no effect of dietary methionine level on broiler chicken meat tenderness and juiciness.

5.2 CONCLUSIONS AND RECOMMENDATIONS

5.2.1 Unsexed broiler chickens aged one to 21 days

The diets used in this study had similar nutrient content levels except for methionine. These nutrient levels meet the nutrient requirements of unsexed Ross 308 broiler chickens. Thus, any differences in responses by the chickens must be due to dietary methionine level. Dietary methionine level did not have any effect on diet DM intake, FCR, growth rate, metabolisable energy intake and nitrogen retention of unsexed Ross 308 broiler chickens aged one to 21 days, possibly indicating that 4g of methionine per kg DM diet is enough for optimal productivity of the chickens aged one to 21 days. Further studies are recommended to ascertain these results.

Results of the present study indicate that dietary methionine level did not affect CP, ADF, NDF and fat digestibilities of unsexed Ross 308 broiler chickens aged 14 to 21 days. However, higher dietary methionine levels of 8 or 9g/kg DM improved DM and ash digestibilities in unsexed Ross 308 broiler chickens aged 14 to 21 days. Dietary methionine level of 7.260g/kg DM optimized broiler chicken dry matter digestibility. Reasons for different responses to dietary methionine levels of 4 up to 9g/kg DM are not clear and require further studies.

Dietary methionine level did not have any effect on live weight of the chickens aged 21 days. However, results of the present study indicate that increasing dietary methionine level from 4 to 9g/kg DM tended to reduce live weights of unsexed Ross 308 broiler chickens aged 7 or 14 days. Thus, dietary methionine levels of 5.29 and 4.99g/kg DM optimized live weights of broiler chickens aged 7 or 14 days. Reasons for differing responses to dietary methionine level are not clear. This requires further studies.

Results of the present study indicate that dietary methionine level did not have any effect on crop, gizzard and small intestine weights and crop, proventriculus and gizzard digesta pH values of unsexed Ross 308 broiler chickens aged 21 days. However, increasing dietary methionine level from 4 to 9g/kg DM affected proventriculus and large intestine weights, gastrointestinal tract and small intestine lengths, and small and large intestine digesta pH values of unsexed Ross 308 broiler chickens aged 21 days. Thus, dietary methionine levels of 6.80, 4.84 and 6.37g/kg DM optimized broiler chickens caecum and large intestine lengths, and large intestine digesta pH.

5.2.2 Male broiler chickens aged 22 to 42 days

The diets used in this study had similar nutrient content levels except for methionine. These nutrient levels meet the requirements of male Ross 308 broiler chickens (McDonald *et al*., 2010). Thus, any differences in responses by the chickens must be due to dietary methionine levels.

The present study indicates that dietary methionine level did not have any effect on diet DM intake of the chickens aged 6 weeks. However, increasing dietary methionine level from 4 to 9g/kg DM improved diet DM intake of male Ross 308 broiler chickens aged 4 and 5 weeks. This might have been due to improved nitrogen retention and live weights of male broiler chickens aged 4 and 5 weeks. Dietary methionine level affected diet DM, CP, NDF, ADF, fat and ash digestibilities in male Ross 308 broiler chickens aged 35 to 42 days. Dietary methionine levels of 6.93, 7.70 and 6.85g/kg DM optimized broiler chicken DM, CP and fat digestibilties. Reasons for differing responses to dietary methionine levels of 4 up to 9 g/kg DM are not clear. Dietary methionine level did not affect FCR and growth rate of male Ross 308 broiler chickens. Results of the present study indicate that dietary methionine level did not have any effect on metabolisable energy intake but increasing dietary methionine level improved nitrogen retention of male Ross 308 broiler chickens aged 22 to 42 days. A dietary methionine level of 9g/kg DM improved live weights of male broiler chickens aged 35 and 42 days. A dietary methionine level of 11.27g/kg DM optimized live weights of broiler chickens aged 42 days.

Dietary methionine level did not affect proventriculus, gizzard, caecum and large intestine weights, caecum, small and large intestine lengths, and crop, gizzard, caecum and large intestine digesta pH values of male Ross 308 broiler chickens aged 42 days. However, dietary methionine level affected crop and small intestine weights, GIT lengths, and proventriculus and small intestine digesta pH values of male Ross 308 broiler chickens aged 42 days. Dietary methionine levels of 6.56 and 7.85g/kg DM optimized broiler chicken crop weights and proventriculus digesta pH values.

Dietary methionine level affected carcass organ weights of male Ross 308 broiler chickens aged 42 days. Increasing dietary methionine level increased chicken breast meat weight. However, there were no clear trends for the other carcass organs. Results of the present study indicate that dietary methionine level did not have any effect on male Ross 308 broiler chicken meat flavour and shear force values. However, dietary methionine level affected meat juiciness and tenderness of male Ross 308 broiler chickens aged 42 days. Thus, dietary methionine levels of 10.09 and 13.32g/kg DM optimized broiler chicken meat tenderness and juiciness values.

CHAPTER SIX

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