Optimal energy to lysine ratio for performance of broilers from day-old to 21 days of age

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DECLARATION OF ORIGINALITY

I, Bilqees Mahomed, hereby declare that this dissertation submitted for the obtainment of the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria is my own work as has not been previously submitted by myself for a degree at any institution.

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Abstract

The seven day period post hatching is considered critical as it is thought to account for 8-10% of the final body weight of broilers at 40 days of age. The digestive capacity of newly hatched chicks is quite low, and increases with age to support growth. By meeting the nutritional requirements of the young chick, broiler performance may be improved throughout the production cycle by means of carryover effects. Not only will this increase the return of investment on feed costs but will also reduce the negative environmental effects that result from broiler production. Despite models using 21 day old birds having proven to yield better estimates of nutritional requirements during the early feeding phase, birds older than 40 days of age are still widely used in models for determination thereof.

This trial was conducted in an effort to identify the optimal dietary energy (metabolisable energy) to protein ratio (expressed on the basis of total lysine) for Ross 308 broiler chicks from 0 to 21 days of age. Birds were provided with 12 treatments comprising of three metabolisable energy (ME) levels (11.31, 12.13 and 12.97 MJ/kg) in combinations with four total lysine (TLys) levels (1.3%, 1.4%, 1.6% and 1.7%). Each treatment was replicated 4 times. Following the experimental feeding phase, all birds were fed the same Grower, Finisher and Post-finisher diets until slaughter at 35 days of age. At day twenty-one of the trial, two male birds were randomly selected from each pen, euthanised and these carcasses were analysed for crude fat, fibre and protein. Body weight, feed intake and mortality were measured on days zero, three, seven, 10, 14, 21, 28 and 35 of the trial.

Optimal broiler growth was found at TLys1.4 with no differences between ME levels. However, at the lowest TLys level body weight (BW) improved with every increase in ME level. Body weight gain (BWG) was similarly favoured by TLys1.4 during the experimental feeding phase but an inversed effect was observed in the carryover phase, where improved BWG was noted at high TLys levels and low ME levels. Feed intake (FI) was more influenced by TLys than ME levels, with increased FI when TLys increased from TLys1.3 to 1.4 at ME 11.30 and 12.13. Resultant 35 day feed conversion ratio (FCR) did not improve beyond TLys1.4 and differences due to ME levels were only observed at the lowest and highest TLys levels (ME 11.30 being the least favourable). No treatment effects were observed for mortality. The calculated performance efficiency factor (PEF) of the broilers in this study was maximised at TLys1.4 with no differences between ME levels; however, these results were matched when TLys1.3 was combined with ME 11.30. Carcass fat was observed to decrease with increasing TLys levels across all ME levels and carcass protein conformed to expected trends by increasing with increased TLys levels. Results based on broiler performance suggest that TLys of 1.4% in combination with ME levels as low as 11.30 MJ/kg may be successfully used in the early feeding phase.

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List of abbreviations

AA	Amino acid
AME	Apparent metabolisable energy
BP	Balanced protein
BW	Body weight
BWG	Body weight gain
CP	Crude protein
dLys	Digestible lysine
EAA	Essential amino acids
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
FI	Feed intake
Lys	Lysine
ME	Metabolisable energy
Met	Methionine
NE	Net energy
NEAA	Non-essential amino acids
NSP	Non-starch polysaccharides
PEF	Performance efficiency factor
TLys	Total lysine
TME	True metabolisable energy

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Chapter 1
Introduction

Optimal broiler production depends on meeting the animals' requirements for growth whilst avoiding wastage associated with production input. Providing dietary energy and protein in quantities similar to those required by the bird has been of interest as a means of reducing the nutritional cost, as well as the environmental pollution associated with broiler production. Nir (1995) observed that broiler body weight (BW) at six days of age had a significant positive correlation to BW at six to seven weeks of age. This finding was supported by the theory that the seven day period post hatching accounted for 8-10% of the BW at 40 days of age and is thus considered critical for broilers (Lilburn, 1998).

The model used to determine energy and amino acid (AA) requirements in the early feed phases is widely practised using broilers older than 40 days of age, despite younger birds (21 day old) having proven to yield better estimates of AA requirements in the Pre-starter and Starter phases (Brito *et al.*, 2011). Wijtten *et al.* (2004a) found that increased balanced protein (BP) levels in the Starter improved broiler growth in that phase, as well as throughout entire growing period. When BP was increased in the Grower phase, following an adequate BP Starter, a lower magnitude of response in broiler performance was elicited. This corroborates the significance of increased BP level in the Starter phase. Carryover effects similar to those observed between BP levels in Starter and Grower phases were also evident between Grower and Finisher phases (Pesti & Fletcher, 1984). Wijtten *et al.* (2004a) observed a long term carryover effect where increased BP in the Starter phase resulted in abdominal fat accretion being significantly lower at slaughter. Increasing the level of BP in the early feed phases may be regarded as an investment since the increase in feed cost may well be exceeded by the profit generated through improved BWG in the subsequent phases of the production cycle.

Improvements in broiler performance traits seen in response to changes in the energy to protein ratio have been partially attributed to the enhanced development of the digestive system (Swatson *et al.*, 2000). Diets with varying combinations of ME and total protein levels resulted in significant changes observed in the digestive organs in broilers from 12 to 24 days of age. These involved increases in gizzard, duodenal, pancreatic, caecal and ileal weights, as well as the digesta holding capacity of the gizzard and small intestine. Treatments with the highest ME to protein ratios per ME level tested yielded the best broiler performance. Lilburn (1998) emphasised that young chicks are metabolically prepared to oxidise fatty acids and thus to utilise dietary fat, provided that fat sources containing high levels of long chain unsaturated fatty acids (vegetable oils). This supported the findings of Swatson *et al.* (2000) that encouraged an increased ME level in the post hatch feeding phase. Direct mechanisms through which changes in the digestive organs affected the performance response were not elucidated, rather the increased rate of digestive organ development and

its possible impact on protein utilisation efficiency is assumed to be responsible for a proportion of this effect (Swatson *et al.*, 2000). This theory may be supported by work conducted by Noy & Sklan (1997) who observed that birds deprived of feed and water in the first forty eight hours post hatch had reduced villus size and enterocytes per villus five to six days later. It was suggested that reduced intestinal function resulted in depressed growth rate of birds over an extended period.

Research on the interaction between dietary ME and dietary AA on broiler performance and carcass traits conducted by Cho (2011), revealed a reliance on the ratio of BP with energy in the diet. The author's findings implied that at higher levels of dietary Lys, more energy is required for optimal broiler performance. Chang *et al.* (2015) examined the effect of decreasing ME levels in combination with BP levels at lower and higher levels recommended by the Ross 308 broiler manual (Aviagen, 2007) under moderate heat stress conditions. The findings of this experiment corresponded with those reported by Cho (2011). Ullah *et al.* (2012) tested combinations of varying levels of ME and Lys inclusion rates at a fixed protein level of 21% in the Pre-starter diet (days 1 to 10 of the productive cycle). These findings demonstrated that increased Lys levels at the higher ME level resulted in optimal broiler performance. The improvement in slaughter weight was thought to be correlated with the superior seven day body weight. It must be noted that the ME levels tested in the above mentioned studies (Cho, 2011; Ullah *et al.*, 2012) were below the recommended levels as specified by both the NRC (1994) and Aviagen (2012).

It is undeniable that broiler performance at time of slaughter can be improved by nutritional intervention during the early feeding phases. The aim of this trial was to identify the ideal combinations of dietary energy and protein levels in the Pre-starter and Starter phases that would optimise broiler performance throughout the broiler production cycle. While an increase in BP level is expected to improve broiler performance, the maximum level that will elucidate a significant response in broiler performance must be specified at the various energy levels it is tested against. Similarly, lower energy levels have been implied to be sufficient to support optimal broiler performance but the minimum level that will suffice for each BP must be identified. Both these parameters (dietary balanced protein and energy) were analysed with focus on the early feeding phase and the carryover effects resulting from them at slaughter in order to better understand the nutritional requirements of broilers from hatching to 21 days of age.

Chapter 2
Literature Review

Energy and protein are the two largest constituents of broiler diets and are also the primary determinants of production cost through their influence on feed cost as well as resultant broiler performance (Cho, 2011). Due to the high cost of synthetic AA available for animal feeds, small changes in inclusion levels result in significant feed cost differences (Viera *et al*., 2016). The use of mature broilers as a model to determine energy and amino acid (AA) requirements in the early feed phases is widely practised, despite younger birds (21 day old) having proven to yield better estimates of AA requirements in the Pre-starter and Starter phases (Brito *et al.*, 2011). It is thus important for further research to be conducted to identify ideal levels of energy and protein for the early feeding phases, in order to improve overall broiler production and reduce feed costs.

2.1 Dietary energy for broilers

2.1.1 Classification of energy

Energy requirements of broilers may be divided into that required for maintenance, such as basal metabolism (thermogenesis and physical activity) and that used for production. The energy contribution of a feedstuff may be represented as gross energy (total combustible energy), digestible energy (DE) (gross energy less the energy lost through faecal excretion), ME (DE less the energy lost through production of gas and urinary products) and net energy (NE) (ME less the energy lost through heat production during digestion and metabolism) (NRC, 1994). Metabolisable energy (ME) or apparent metabolisable energy (AME) may be corrected for endogenous energy loss, to give true metabolisable energy (TME) (Sibbald, 1976). A further correction made for nitrogen retention of the animal yields TMEn. This is thought to be the most accurate estimation of the energy content of a feedstuff due to the variables it accounts for. However, due to the relatively small contribution of endogenous loss to overall energy loss, AMEn is more commonly used as an estimate of ME (Parsons, 1982).

The shortcomings of using ME on an energy evaluation platform include the lack of differentiation between efficiencies of different energy generating precursors and disregard of the effect of the animals' age/physiological status on the ME value for individual feedstuff, as well as the effect of the level of feeding on energy utilisation efficiency (Batal & Parsons, 2004). NE values provide the best estimate of the actual energy contribution of a feedstuff, as it does account for the differences in efficiency of utilisation of energy precursors, but direct measurements thereof are expensive and time consuming (Pirgozliev *et al*,. 2011).

Indirect methods include use of prediction equations based on DE/ME values and chemical characteristics of the relevant diets (Noblet, 2010).

2.1.2 Sources of dietary energy for broilers

Broilers may derive energy from carbohydrates, protein and fats (NRC, 1994). Carbohydrates, specifically the starch component, are the main energy contributor to dietary energy (Cho, 2011). Starch may be present either as amylase (consisting of linear glucose chains, which are not as readily cleaved by amylase) or amylopectin (consisting of linear and branched glucose chains, making it more susceptible to enzymatic cleavage). Some polysaccharides exist that are not of starch origin; called non-starch polysaccharides (NSPs). The enzymes required to digest NSPs are not naturally secreted by monogastric animals, thus nullifying their possible energy contribution. Water soluble NSPs consist of ß-glucans, arabinoxylans and fructans, all of which have a marked anti-nutritional role of increasing digesta viscosity in the small intestine, which adversely affects its absorption capacity (Iji, 1999). NSPs also consist of dietary fibre, which is fermentable but indigestible carbohydrates. Due to the water insolubility of dietary fibre, intestinal digesta viscosity is unaffected by its presence (Hetland *et al.*, 2004).

Lipids are the most energy dense feed component and are most efficiently metabolised, possibly due to a relatively lower heat increment during digestion (Classen, 2013). Whilst hard fats (tallow and lard) are not utilised by young birds, vegetable fats are readily utilised and can be included in the diet up to 3-4% before affecting pellet quality; an additional 2-3% may be sprayed on onto the already-formed pellet. Fats are commonly found as triglycerides and sometimes as free fatty acids. Saturated fatty acids may be formed *de novo*, with the exception of linoleic and α-linolenic acids, which are referred to as essential fatty acids. Since fatty acids are not excreted in the urine, their ME values are determined by the rate at which they are absorbed from the digestive tract (Kleyn, 2013). Dietary protein may be used as glucose precursors, but this is less efficient as protein must first undergo amino acid (AA) catabolism to supply energy; which has an associated energy cost of excretion of excess nitrogen as uric acid (Cho, 2011).

2.2 Dietary protein for broilers

Broiler production centres on the conversion of feed protein to animal protein (meat production). Following energy, protein is the second largest constituent of broiler diets and is the most expensive component thereof. Apart from the financial implication of excess protein, the environmental impact of nitrogenous waste excreted from livestock must be accounted for (Nasr & Kheiri, 2011). The protein value of a feedstuff is usually depicted as crude protein (CP), which is calculated by multiplying its nitrogen percentage by a factor of 6.25. This is an inaccurate estimate as it does not account for the different nitrogen:

protein ratios that exist across feed ingredients and unlike true protein, CP is not solely based on the AA contribution of the feedstuff, but also on the non-protein nitrogen that may be present (Mariotti *et al.*, 2008). Protein requirements in broilers actually signify the requirement of amino acids (AA), the monomers of protein. Factors determining this requirement include the level of bird productivity (growth and egg production) as well as broiler maintenance (AA used in production of body components and in various metabolic pathways) (NRC, 1994). Malheiros *et al.* (2003) found that protein concentration was most consequential when the effects of low levels of dietary fat, energy and protein on broiler growth were evaluated.

2.2.1 Amino acids in broiler diets

Amino acids are compounds of carbon, hydrogen and nitrogen (sometimes also containing sulphur and/or phosphorus), that are covalently bonded by peptide bonds to form proteins. These may be either of dietary or animal (endogenous) origin. Free forms of AA and small peptides may be found in AA pools within the animal's body, at concentrations reflecting its AA balance. Of the 22 different known AA, 11 are classified as essential AA (EAA). This is based on their lack of or limited *de novo* syntheses which necessitates their dietary supplementation (Leeson & Summers, 2001). For poultry, eight of the EAA are considered critical, as they are not present in significant amounts in a typical diet. Non-essential AA (NEAA) are readily formed in the body, thus dietary requirement is minimal or null. The classification of amino acids for broilers is shown in Table 2.1.

Table 2.1 Classification of amino acids for broilers (adapted from Leeson and Summers, 2001)

Essential	**Non-Essential**
Lysine	Glutamine
Methionine	Alanine
Cysteine	Glutamic acid
Isoleucine	Aspartic acid
Arginine	Asparagine
Threonine	Hydroxyproline
Tryptophan	Glycine
Valine	Serine
[1]Histidine	Proline
[1]Phenylalanine	
[1]Leucine	

[1] Non-critical EAA

2.2.2 Amino acid digestibility

Amino acid digestibility refers to the fraction of dietary AA which is retained following ingestion (not excreted in the faeces) and can be calculated as the difference between AA intake and faecal output as a fraction of intake. Since the evaluation of AA availability is expensive and time consuming (Garcia, 2006), AA digestibility is used as an estimate of availability. This also allows for diets to be formulated with more precision with regard to meeting nutritional requirements. Feed ingredients with low levels of digestible AA are often financially wasteful and contribute to heightened levels of nitrogen in the faeces through the excretion of excess AA (Lemme *et al.*, 2004). When such feed ingredients constitute large proportions of the diet, broiler performance can be more difficult to accurately predict due to the limited availability of AA (Fernandez *et al.*, 1995). Nasr & Kheiri (2011) examined the difference between diet formulations based on total AA versus digestible AA inclusion on broiler carcass yield and found that breast meat yield and abdominal fat accretion were significantly higher when digestible AA values were used. This result was attributed to a higher AA intake from these diets.

2.2.3 Amino acid imbalances

Single AA deficiencies in broiler diets (up to 50% deficient) resulted in reduced growth rate and feed intake (FI); this is especially prominent with EAA (Okumora & Mori, 1979). Single AA excesses are more dangerous, with Met being the most toxic, resulting in depressed growth and FI. Excesses of certain AA may limit the efficiency of the use of the first limiting AA. Antagonism between AA may also be present, wherein excess of a certain AA increases the requirement for another, metabolically similar AA, as seen in birds with high levels of Lys elevating renal arginase activity and thus the Arg requirement (Nesheim, 1968). Such factors may contribute to the impaired growth characteristics that have been noted in response to AA excesses (Han & Baker, 1993), however, no thermogenic difference was observed in diets containing imbalanced protein (Macleod, 1997).

Variations in protein quality that result in AA imbalances may have an effect on energy utilisation in broilers. Resultant effects may be seen in BWG and nitrogen retention, without any associated changes in feed or nitrogen intake. This was demonstrated by findings of Nieto *et al.* (1995), where supplementation of a Met deficient broiler diet with DL-Met resulted in increased weight gain, and nitrogen and energy retained as protein. When the same diet was supplemented with L-Lys, weight gain, and nitrogen and energy retained as protein decreased. This suggested that supplementation of the first limiting AA can improve the quality of dietary protein, as well as the efficiency of dietary ME utilisation and thus growth, however, over-supplementation of an AA can lead to a further imbalance in the AA profile, which may reduce daily weight gain and nitrogen retention. Supporting results were found by Macleod (1997), which indicated that protein quality played a role in AA imbalances. Low Lys levels from a balanced protein (BP) were shown to yield a

higher TME intake in broilers from 21 days of age, when compared to high Lys levels from an unbalanced protein. Protein retention was positively correlated with Lys concentration (intake) but negatively so with Lys to CP ratio, thus with lower protein quality, a higher protein retention per gram of Lys was observed. Heat production, measured by indirect calorimetry, was shown to correlate with Lys intake, Lys to CP, as well as Lys to TME ratios. The change in body composition resulting from a difference in CP and TME ratio was not accompanied by a change in the regulatory heat increment.

2.2.4 Balanced protein for broilers

Despite the importance of EAA supplementation, it alone is not enough to optimise broiler performance. Pesti (2009) demonstrated that even with the supplementation of sufficient EAA necessary to support growth, chickens fed low-protein diets did not perform positively as was expected, and instead accrued excessive fat instead of lean muscle; thus, the requirement for NEAA as well as EAA was hypothesised. It has been acknowledged that the AA requirement of poultry is affected by genetic, nutritional and environmental factors (Wijtten *et al.*, 2004a), necessitating the need for a common basis for AA requirements to be quantified. The concept of ideal or balanced protein (BP) is theoretically that which precisely meets an animal's AA requirement, by supplying the correct balance of EAA and sufficient nitrogen for the *de novo* production of NEAA without any deficiencies or excesses (Emmert & Baker, 1997). Since requirements for EAA have been described in values relative to Lys, all AA within BP are equally limiting, so although the absolute values of the AA required may change, the ratio between these values is relatively constant (Wijtten *et al.*, 2004b).

Multiple factors have led to the level Lys being used as the reference AA relative to which other EAA are given proportional values. These include Lys often being the second limiting AA (limiting being that AA which is most deficient relative to others), extensive studies having been conducted on Lys, analysis of Lys content in feedstuffs being relatively uncomplicated and the function of Lys being solely for maintenance and accretion of protein (not used as a precursor for NEAA synthesis) (Emmert & Baker, 1997; Plumstead, 2005). Theoretically, the BP concept indicates the possibility of maintaining broiler performance at lower dietary CP levels, by inclusion of sufficient and balanced essential AA. However; this did not hold true in all trials using low protein levels. In these cases, lower protein diets did not yield the same performance as the control diets. Such results could be attributed to the modern broiler strain having a high requirement of non-essential as well as essential AA (Lemme *et al.*, 2003).

2.3 Energy to protein ratios in broiler diets

2.3.1 Factors affecting response of broilers to dietary energy and amino acid levels

Work by Classen (2013) reviewed multiple aspects of broiler management as having an influence on broiler response to energy. When focusing on energy to protein ratio, the author proposed that each component be treated as a possible limiting factor so that whichever was first limiting would be of primary importance for that specific situation. This led to the conclusion that the ratio of consequence is in fact that of dietary energy to the level of digestible ideal essential AA, provided that minimum protein levels are available to support sufficient synthesis of non-essential AA.

Differences in response between sexes of broilers were observed by Wijtten *et al.* (2001) who found that higher dietary BP levels led to a higher response in male birds than females, where BWG in males increased in response to up to 130% Dutch CVB standard and females only up to 110 to 120% Dutch CVB standard. Feed conversion improved in response to increasing dietary BP level in both sexes, but to a higher degree in males. Male broilers have a higher potential for improved BWG and feed conversion efficiency, and thus a higher AA requirement to fulfil said potential. A further finding by Baker (2009) was that instead of an overall increase in AA requirements, the requirement specifically for Lys was higher in male chicks than in females.

The effect of broiler age may be attributed to the development of the digestive system in newly-hatched chicks; which is continuous throughout the first few weeks post-hatch. This can be assumed from performance results as reported by Brito *et al.* (2011), in which ileal digestibility of cecectomised broilers of seven days of age provided a better estimate of 21 day old broiler AA requirement than that of broilers of 42 days of age. Protein gain as a proportion of BWG is reduced with progressive age and BW, thus a corresponding reduction in AA and protein requirement exists for older, heavier birds (Baker, 2009). This finding is supported by Wijtten *et al.* (2004b), wherein increasing BWG and decreasing FCR responded linearly to increasing dietary BP levels in broilers from 14 and 34 days of age, but exponentially in broilers from 28 to 41 days of age.

Feed form, pellet quality and particle size of feed ingredients used in finished feed are all implicated as indirectly affecting broiler performance through their effect on FI. If these can be controlled, the aforementioned response may be improved to an extent. Work conducted by Greenwood *et al.* (2005) indicated that BWG of broilers responded positively to higher dLys levels when feed was provided in pellet form rather than in form of a mash. A similar relationship was seen between feed form and feed efficiency (FE), with the additional finding that dLys deficient diets in the pellet form amplified the deficiency effect on FE more so than the mash form. Increased feed and energy intake was observed with pelleted feed. The increased potential response of BWG and FE to dLys suggested that pelleted feed should be used as the

model for determining dLys requirements for broilers. Findings by Lemme *et al.* (2006) showed that despite higher final BW having resulted from diets containing good quality pellets, BWG of poor quality pellets could possibly be equivalent, provided they contained higher BP levels. In agreement with the findings of Greenwood *et al.* (2005), FI in birds offered mash was lower than in birds offered pellets, resulting in a decreased BWG. If digestible Lys levels were increased, the decrease in FI of mash became even more pronounced than that of pellet diets. These observations may be attributed to the increased energy cost of consuming finer particles. The importance of pellet quality should not be ignored when aiming to optimise nutrient intake while reducing production costs.

2.3.2 The effect of energy to protein ratios in broiler diets

2.3.2.1 Feed and nutrient intake

High energy feeds were often assumed to reduce FI (Liu *et al.*, 2016). This has allowed for AA recommendations to be provided as a function of the energy value of the diet (i.e. gram per MJ ME). The implication of this was that for a higher energy density, a higher AA density was necessary to compensate for the associated reduction in FI in order for optimal broiler performance to be achieved (Lemme, 2007). Work by Bellaver *et al.* (2002) has demonstrated that FI from days 1 to 21 of the growing period decreased as the dietary ME level increased from 12.55 to 13.39 MJ/kg, provided dietary AA was balanced relative to a digestible Lys level of 10.2 g/kg and higher. Weight gain responses for ME densities of 12.97 and 13.39 MJ/kg indicated that the optimum response was achieved at the BP levels between 9.2 and 12.2 g/kg. For optimum response to have resulted from the 12.55 MJ ME/kg diet, a higher level of BP would have been required (weight gain showed an increasing trend even with the highest digestible Lys to ME ratio used in this experiment). Results of a similar experiment conducted by Plumstead (2005) concurred with these results, where it was shown that a higher digestible Lys to ME ratio was required at decreasing ME densities for broiler performance to be maintained. Therefore, it may be concluded that the increase in FI associated with a lower energy density of the diet alone is not sufficient to allow for adequate AA intake to meet relevant broiler requirements (Lemme, 2007).

Despite the regulatory effect dietary energy is assumed to have on FI, its influence on BWG is considered indirect due to the more significant effect resulting from the level of dietary protein, which in turn is controlled by FI. Lemme *et al.* (2005) tested combinations of dietary ME and BP AA levels at various fractions of the Aviagen Ross Management Guide recommendations across each feed phase. From day zero to 46 of the trial, FI was shown to increase non-linearly with increasing dietary BP levels and decreasing dietary ME densities. At 95% ME recommendation, energy intake was shown to have been maintained through increased FI, but at lower dietary ME levels, FI could not be sufficiently increased to maintain

energy intake. BWG increased non-linearly with increasing dietary AA density, as was seen across all energy densities, however higher weight gains were seen with lower energy densities where FI increased, especially in the Starter phase. Another favourable response to increasing dietary AA density observed was a reduction in the FCR. Decreasing ME levels resulted in favourable weight gains. This effect was further improved with increasing AA levels, which in turn is increased by the energy-driven increased FI. Relatively higher dietary AA levels and lower ME levels relative to the Aviagen recommendation were shown to yield the best broiler performance, thus implying that as dietary ME is lowered, dietary AA should not be reduced, or at least not to the same extent. Faulkner (1993) had seen a similar trend in FI when feeding decreasing Lys levels in combinations with and without sucrose supplementation. While birds attempted to increase Lys intake through increasing their FI, the addition of dietary sucrose proved to impede their efforts. Explanations for this reduction in FI included the birds having been unable to cope with the increased heat increment resulting from the high energy intake as well as a reduced body size (physical capacity) resultant from the low Lys intake. In further agreement with Lemme *et al.* (2005), Faulkner (1993) observed an increase in growth rate with increasing dietary Lys levels until the point at which it reached a plateau – this was thought to be due to insufficient dietary energy. Increasing dietary energy levels relative to protein levels also reduced growth rate through increased fat deposition as well as heat production.

More recent work conducted by Plumstead *et al.* (2007) negated the effect of dietary energy density on the level of FI in broilers. The first experiment tested combinations of three dietary ME levels varying from 12.55 to 13.39 MJ/kg with four dietary CP levels from 21.9% to 26.9%. The AA profiles were pre-set to contain the ideal ratio of EAA relative to digestible Lys (4.8% CP). Results at 21 days depicted an increase in BWG and a reduction in adjusted FCR, in response to increasing levels of CP at the lowest ME level (12.55 MJ/kg) as well as to an increase in dietary ME from 12.55 to 12.97 MJ/kg. These responses were observed despite the lack of associated changes in FI. Neither BWG nor FCR implied consistency in the relationship between BP and ME leading to the hypothesis that beyond the ME 12.55 MJ/kg, BP elicited a response independent of the ME level. The second experiment by Plumstead *et al.* (2007) examined the response elicited by graded inclusion rates of digestible Lys at low and high balanced CP dietary inclusions. BWG at 20 days of age increased linearly with an increase in dietary CP provided the AA profile was balanced. Since dietary protein was not shown to influence FI, the improvement in BWG was assumed to be a result of a higher AA intake. It was further proposed that differences in levels of FI in previous studies may be attributed to differences in formulation techniques, ages of the birds and the range of dietary ME tested.

Feed intake showed a positive correlation to dietary BP independently of dietary energy density when Madsen *et al.* (2010) examined the effect of graded BP levels (80%, 90%, 100% and 115% of Aviagen recommendation) at energy levels of 95% and 100% of Aviagen recommendation. Experimental diets were introduced from day eleven of the trial. Although FI increased at the lower energy inclusion level, overall

energy intake was lower than that observed in the higher energy diet and increasing BP levels resulted in decreased FI at both energy levels. Thus, energy intake was reduced but protein intake was not, as the higher protein diets were able to fully compensate for the lower FI. Weight gain increased with increasing BP levels; however, the differences between energy levels became progressively less pronounced as the BP level increased. FCR was unfavourable at the lower energy level and improved with increasing BP levels (even at that above the recommended BP level).

2.3.2.2 Broiler performance

Early access to feed and water to newly hatched chicks has been shown to improve BW throughout the growing period. This effect was attributed to the stimulation of growth and activity in the digestive tract (Noy & Sklan, 1997). Swatson *et al.* (2000) tested the hypothesis that improvements in broiler performance traits seen in response to changes in the energy to protein ratio may be partially attributed to the enhanced development of the digestive system. The authors observed that diets with varying combinations of ME and protein levels between 11 and 14 MJ/kg, and 250 and 500 g/kg, respectively, resulted in significant changes observed in the digestive organs in broilers from 12 to 24 days of age. These involved increases in gizzard, duodenal, pancreatic, caecal and ileal weights, as well as the digesta holding capacity of the gizzard and small intestine. Treatments with the highest ME to protein ratios per ME level tested yielded the highest BWG, FI and feed conversion efficiency, with the overall highest BWG and FCR resulting from a combination of 13 MJ/kg and 250 g/kg protein. Direct mechanisms through which changes in the digestive organs affected the performance response was not elucidated, rather the increased rate of digestive organ development and its possible impact on protein utilisation efficiency is assumed to be responsible for a proportion of this effect.

Research on the interaction between dietary ME and dietary AA on broiler performance and carcass traits conducted by Cho (2011), revealed a reliance on the ratio of BP to energy in the diet. Dietary ME was provided at 11.30, 11.85, 12.41 and 12.97 MJ/kg across three ileal digestible Lys levels (between feed phase specific ranges) throughout the grow-out period. At the highest Lys level, increasing energy content of the diet yielded increasing values for body weight, FE as well as carcass and breast meat yield, despite no significant difference observed in FI. This indicated that at higher levels of dietary Lys, higher levels of dietary energy were required to optimise broiler performance. Intermediate Lys levels were shown to be adequately matched by all ME levels provided, thus no effect of ME could be seen in the measured responses. At low levels of dietary Lys, increasing ME resulted in lowered FI, growth rate and body weight, as expected with an increasing imbalance between these dietary components. BWG response to increasing dietary BP to ME ratio beyond the Starter phase has been observed at ME levels of 12.55 MJ/kg and higher, while lower ME levels do not show a significant difference in weight gain. FI also significantly differed

between energy levels fed, showing a decreasing trend with increasing dietary ME. Feed conversion improved with both increasing energy level as well as increasing protein to energy ratio, with better responses at moderate to low energy levels. Similar results were found when Liu *et al.* (2016) fed five diets with various ME to CP ratios from days seven to 28; diets with low protein concentrations yielded lower weight gains and FI. The effect of ME to CP ratios on BWG and FCR appeared to be quadratic, indicating a need for both protein and energy for efficient muscle deposition. This is in agreement with the earlier findings of Emmans (1987), who theorised that birds eat to meet their requirement of the first limiting nutrient in the feed.

Work by Ullah *et al.* (2012) demonstrated an overall decreasing trend in FI with increasing ME levels, and an increase in FI with increasing Lys levels at the highest ME level, when feeding varying levels of ME (11.51 to 11.92 MJ/kg) and total Lys inclusion rates (1.3% to 1.5%) at a fixed protein level of 21% from days 1 to 10 of the production cycle. Birds consuming the diet with the highest ME density and Lys inclusion level during the Pre-starter phase had the highest FI in the last three weeks of the production cycle, which resulted in the most unfavourable FCR. The best FCR was achieved by a combination of 11.92 MJ/kg ME and 1.3% Lys, this FCR value being non-significantly different from that resulting from combinations of 11.72 MJ/kg ME and 1.4% Lys, and 11.92 MJ/kg ME and 1.4% Lys. Maximum dressing percentage, digestive tract weight and intestinal length (all having non-significant difference between treatments) as well as slaughter weight (significantly higher), were also achieved with the combination of 11.92 MJ/kg and 1.4% Lys, the latter of which thought to be correlated with the superior seven day body weight as having resulted from said diet. It must be noted that the ME levels tested in the above mentioned studies (Cho, 2001; Ullah *et al.*, 2012) were below the recommended levels as specified by both the NRC (1994) and Aviagen.

2.3.2.3 Carcass composition

Along with a higher final body weight, growth rate and improved FCR, genetic selection of the modern broiler has also resulted in a higher fat deposition potential (Baéza & Le Bihan- Duval, 2013). Comparisons were done between the carcass yield and composition of broilers as reared in 1970 (ARBC strain) versus “modern broilers” (Ross 308), once in 1991 and again in 2001 (Havenstein *et al.*, 1994, 2003). The effect of dietary improvement over time was accounted for through the inclusion of both 2001 and 1997 diets as levels in the experimental design, and was confirmed with Ross 308 birds having had 25-33% heavier carcasses on the 2001 diet than on the 1970 diet. In contrast, ARBC bird BW improved only by 12-13% on the 2001 diet, with the implication that the older strain did not have the genetic potential to utilise the improved diet (strain x diet effect). When comparing both strains on the same diets, Ross 308 broilers presented consistently higher average carcass weights of 535% and 453% of the ARBC carcass weights on the 2001 and 1970 diets respectively, at 43 days of age. A significant increase in total breast meat yield was

also observed when comparing the results of the modern strains across the two experiments, with 2001 birds having approximately 6% more breast meat compared to the 1991 strain (ACRBC) despite being only a decade apart. Both studies reflected a lower percentage of body composition comprising of the heart and lungs for the modern strains, as well as relatively higher abdominal fat accretion than that observed for the ACRB strain. Of these differences observed between the ACRBC and Ross 308 broiler strains, 85 to 90% may be attributed to genetic selection, with the remaining fraction attributed to the improvement in the diet (Havenstein *et al.*, 2003). Similar results were seen by Zhao *et al.* (2009) when comparing carcass weight, carcass yield and fat accretion in the Arbor Acres commercial line with that of a Chinese unimproved line (The Beijing-You). It was further noted that performance differences between breeds decreased with decreasing nutrient density, while carcass quality differences decreased with increased nutrient density.

A high fat accretion is associated with an inefficient usage of dietary energy, as it negatively influences carcass yield, consumer acceptability and thus economic return (Emmerson, 1997). Of the nutritional factors that are able to manipulate the degree of fat accretion, energy and protein levels in the diet have been noted as the most effective. A reduction of the energy level in broiler diets from 13.39 to 12.55 MJ/kg from 21 to 42 days of age (Kassim & Suwanpradit, 1996b) and from 13.50 to 12.80 MJ/kg from 18 to 52 days of age (Rabie & Szilyagi, 1998) resulted in a significant reduction in abdominal fat without any effect on other performance traits.

Female broilers between 21 and 42 days of age fed diets with significant differences in energy to protein ratios were shown to influence body composition by altering the rate of fat deposition, without affecting the regulatory heat increment (Macleod, 1990, 1991 and 1992). Linear correlations were seen between dietary CP to AME ratios and carcass protein: fat ratios, carcass protein and carcass fat. Results of a later experiment conducted using male broilers confirmed these findings, where the change in the heat increment between different dietary Lys levels were solely attributed to the resultant change in body composition that was associated with protein and fat accretion (Macleod, 1997). The unchanged heat increment indicated that the increased energy intake did not result in a higher proportion of energy lost through heat, but was rather channelled towards building body components such as fat and protein. Therefore, feed was being efficiently utilised for growth. Increasing dietary AA levels corresponded with increasing breast meat yield and reduced abdominal fat accretion, with maximal meat yield in response to AA supply resulting from a higher AA to energy ratio. Energy above that required for said meat yield would only be stored as body fat (Lemme *et al.*, 2005). This was illustrated in an earlier experiment by Lemme *et al.* (2003) where the highest ME level tested (13.18 MJ/kg) resulted in an increase in breast meat yield and a decrease in abdominal fat accretion, proportional to dietary AA, reaching a maximum response at apparent faecal digestible (AFD) Lys to ME ratio of 8.53 and 8.96 g/MJ, respectively, while decreasing at higher

ratios. Body composition can thus be manipulated by changing not only the amounts of dietary energy and protein, but also by the ratio of protein to energy that is present in the diet.

Fan *et al.* (2008) examined the effect of lowering the dietary ME level from 12.13 to 11.30 MJ/kg in ducks between 14 and 42 days of age and found a significant reduction in abdominal fat relative to body weight, without any change in breast or leg muscle proportions. It may thus be deduced that by lowering the level of dietary ME, the efficiency of dietary energy use may be increased through the reduction of fat accretion. Other studies have also shown that low protein diets increased abdominal and total body fat accretion (Kassim & Suwanpradit, 1996a; Collin *et al.*, 2003), while diets with protein levels above that recommended by the NRC resulted in a lower total body fat accretion (Yalcin *et al.*, 2010). Changes in dietary ME and protein density in diets from day 11 of the growing period were examined by Madsen *et al.* (2010) with results indicating that carcass yield was unaffected by the difference in dietary ME level between 95% and 100% of Aviagen recommendation, but responded positively to increasing levels of BP. The lower ME level and higher BP level combination was shown to reduce abdominal fat accretion and increase breast meat yield implying that current balanced recommendations may not be fully exploiting the genetic growth potential of the modern broiler.

Carcass composition changes noted by Dozier *et al.* (2007) included a 0.1% increase in breast meat yield with a reduction in ME levels from 13.56 to 13.14 MJ ME/kg, as well as a reduction in abdominal fat pad accretion; breast meat yield was also observed to increase from moderate to high AA density. Reduction of AA density had the opposite effect on breast meat yield and also reduced CP, Lys and total sulphur containing AA intake per unit BWG and breast meat yield. The increase in AME levels from 13.47 to 13.85 MJ ME/kg, as tested in a separate experiment, reduced carcass yield and elevated abdominal fat accretion. Breast meat yield was improved with increasing dietary AA levels and moderate ME, with high AA density diets resulting in a higher carcass yield than diets formulated to moderate levels of both AME and AA.

From an individual AA perspective, Met or Lys deficient diets, and diets with Arg levels above the NRC recommendation resulted in increased abdominal fat accretion while increasing levels of Met and Lys were shown to reduce abdominal fat accretion (Grisoni *et al.*, 1991; Corzo *et al.*, 2003, 2006; Xie *et al.*, 2006; Nasr & Kheiri, 2011). Relative to carcass yield of other muscles, breast meat is significantly reduced by low dietary Lys levels (Tesseraud *et al.*, 1996). High dietary Lys levels in the Starter phase (days 1 to 14 of age) in broilers resulted in higher breast meat yield at slaughter weight, but no effect was seen in response to increased Lys levels from days 15 to 49 (Holsheimer & Ruesink, 1998). Abdominal fat deposition may thus be reduced by increasing dietary Lys and Met levels, and preventing excessive Arg levels throughout the growing phase, while investing in increased Lys density in the early growth phase may be remunerated through improvement in breast meat yield at slaughter.

Contradictory results were seen when the effect of increased digestible Lys levels across each feed phase in both male and female broilers was investigated by Tavernari *et al.* (2009). Across all feeding phases, both sexes were fed diets containing 92.5%, 100% and 107.5% of the digestible Lys requirements as stipulated in the Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2000). No significant differences were seen in body compositions with regard to dry matter or fat percentage between the different levels of digestible Lys across any of the feed phases, apart from 42 day old females having a lower carcass protein level in the treatment providing the highest digestible Lys. Body fat accretion did not differ significantly between treatments and protein deposition response was seen only in seven week old males. The lowest digestible Lys level resulted in significantly lower protein deposition at six weeks of age, with no significant difference between 100% and 107.5% of the given requirement levels. At seven weeks of age, 92.5% digestible Lys yielded significantly higher protein deposition than that achieved by 100% of the given requirement level (the higher protein inclusion level was not statistically different to the other two levels). Reasons for the lack in response of body composition to dietary Lys levels were attributed to the possibility of weekly intervals not adequately reflecting the response and the random sampling of just two birds per experimental unit increasing the coefficients of variation. Similar results were found by other scientists (Summers & Leeson, 1985; Eits *et al.*, 2002), where higher Lys levels in the Starter phase did not reduce body fat accretion.

2.4 Early nutrition for broilers

2.4.1 Pre-starter and Starter diets for broilers

The importance of early access to feed for newly hatched chicks has been strongly emphasised by Noy & Sklan (1997). Chicks deprived of feed and water in the first 36 hours post hatch weighed 100-200 g less than chicks given immediate access to feed at forty days of age. Lilburn (1998) suggested that the primary objective of the Pre-starter diet be to optimise the metabolic homeostasis of the flock, following which a diet to optimise maintenance and production be introduced.

According to the Aviagen Ross 308 manual (2014), broilers expected to reach a target weight less than 2.5 kg should be fed following a three-phase feeding programme: Starter from days zero to 10; Grower from days 11 to 24; Finisher 1 from days 25 to 39 and Finisher 2 from day 40 to slaughter. The recommended energy level for the Starter phase is 12.55 MJ/kg, at crude protein (CP) level of 23%. Individual amino acids (AA) as recommended by Aviagen (2014) are shown in Table 2.2. Also depicted are the NRC (1994) recommendations for “meat type chickens” which are split into similar feeding phases (only one Finisher diet), with slightly different time periods of zero to three weeks, three to six weeks and six to eight weeks. The energy level for the phase is somewhat higher relative to the Aviagen recommendations, at 13.39 MJ ME/kg, while the CP level recommendation remains the same at 23%.

Table 2.2 Amino acid recommendations (as % of diet) for meat type chickens from 0 to 3 weeks (adapted from NRC, 1994 and Aviagen Ross 308 broiler manual, 2014)

	NRC 1994	Aviagen 2014	
Amino acids	**Total**	**Total**	**Digestible**
Arginine	1.25	1.52	1.37
Isoleucine	0.80	0.97	0.86
Leucine	1.20	1.58	1.41
Lysine	1.10	1.44	1.28
Methionine + Cysteine	0.90	1.08	1.95
Methionine	0.50	0.56	0.51
Threonine	0.80	0.97	0.86
Tryptophan	0.20	0.23	0.20
Valine	0.90	1.10	0.96

Nutrient recommendations for the Starter phase are heavily based on research conducted on broilers between seven and 21 days of age, thus completely disregarding the influence of yolk absorption as well as the progressive development of the digestive tract in the post hatch period (Garcia, 2006). An alternative feed phase programme includes a Pre-starter phase that is fed from placement of chicks until a predetermined age, with the Starter phase commencing thereafter (Ullah *et al.*, 2012). Benefits of a Pre-starter phase include improved flock uniformity, higher growth rates within the first seven days of age and higher livability throughout the production cycle (Garcia, 2006). Given the limited FI potential of the young chick, a more nutrient dense diet such as the Pre-starter could positively impact the overall production cycle.

2.4.2 Phase-specific and carryover effects of increased dietary balanced protein levels

Having seen an improvement in response to dietary protein in feed phases that were preceded by protein deficient diets (Pesti & Fletcher, 1984; Kidd *et al.*, 1998; Eits *et al.*, 2003), the effect of adequate dietary protein levels (Schutte, 1996) were tested against higher levels in the Starter, Grower and/or Finisher phases (Wijtten *et al.*, 2004a). Increased BP levels in the Starter increased BWG and feed conversion efficiency of that phase, and also resulted in increased BWG and FI in the subsequent Grower phase and continued across the entire growing period. Additional effects of increased BP in the Starter phase that was observed in the second experiment included a lower magnitude of the response in BWG and feed conversion ratio (FCR) to increased BP levels in the subsequent Grower phase. Despite a higher BWG resulting from the high BP Grower following an adequate BP Starter (compensatory growth), BW was still lower than that resulting from the high BP Grower following the high BP Starter. This indicated the significance of the increased BP level in the Starter on broiler performance beyond the Starter phase.

Increased BP levels in the Finisher improved weight gain and feed conversion efficiency in the Finisher phase until slaughter. Carryover effects similar to those observed between BP levels in Starter and Grower phases resulted between Grower and Finisher phases, where high BP levels in the Grower resulted in a diminished response of BWG and FCR to high BP levels in the Finisher phase. Although these finding were not statistically significant, they were consistent between experiments conducted and with results noted in previous experiments (Pesti & Fletcher, 1984). This reduction in feed efficiency may be explained by a compensatory increase in fat deposition in the Finisher phase (Gous *et al.*, 1992) and an increased maintenance requirement due to the higher BW resulting from the Grower phase. Changes in carcass composition in response to the increase BP levels in all feeding phases were noted, namely a non-significant increase in breast meat yield and reduction of abdominal fat deposition in the Starter phase, higher breast meat yield and lower abdominal fat accretion persisting in the Grower phase, and a higher breast meat yield in the Finisher phase. A long term carryover effect from the Starter phase was abdominal fat accretion being significantly lower at slaughter. Increasing the level of BP in the early feed phases may be regarded as an investment since the increase in feed cost may well be exceeded by the profit generated through improved BWG in the subsequent phases of the production cycle (Zubair & Leeson, 1996).

Conclusion

The period following hatching is crucial to the production cycle as it creates a foundation for later broiler performance. BW at seven days has been shown to have a strong, positive correlation with slaughter weight. Much of the improvement in broiler performance resulting from improved nutrition in the early feeding phases is attributed to enhanced development of the digestive system. Energy and AA recommendations as published by Aviagen and the NRC have often yielded inferior results when tested against lower energy and higher AA combinations. The importance of dietary balanced protein has been highlighted as a possible alternative to high total protein inclusion rates, for reducing feed cost. An increase in profit may also be realised through correct AA ratios improving broiler performance, as high levels of ideal protein in the early phase of broiler nutrition positively influences performance during later phases. The level of dietary energy has been thought to influence FI, but this does not always hold true. It has been hypothesised that broilers consume feed at a level so as to maintain energy or AA intake, depending on which is first limiting. The ratio at which protein and energy are present in a diet has also been shown to affect broiler performance, especially in terms of BWG, FE, breast meat yield and fat accretion. Carcass composition changes observed in response to nutrition include a decrease in fat accretion and an increase in breast meat yield with decreasing energy and increasing AA levels. Responses of broilers to altered dietary energy and protein content may differ according to the sex, age and physical feed characteristics amongst other factors.

Chapter 3
Material and Methods

3.1 Experimental design

This trial was conducted at the Daybreak Trial facilities in Sundra (South Africa). The trial house was divided into four blocks, each containing 12 pens, with one replication per treatment per block. There was a total of 12 treatments, comprising of a randomised block design with three levels of metabolisable energy (ME) and four levels of total lysine (TLys). Combinations of four TLys treatments and three ME levels were randomly allocated to each block. The variables analysed were BW, BWG, FI, FCR, performance efficiency factor (PEF) and mortality. These were respectively calculated from the following measurements: bird counts, initial body weight, body weights, feed weighed in and feed weighed out and mortality records. The relevant measurements were conducted on days zero, three, seven, 10, 14, 21, 28 and 35 of the trial, with corrections being done for mortality at every weighing interval.

3.2 Animal husbandry

Ross 308 chicks were randomly selected, counted into groups of 60, weighed and placed into pens at day zero. Chicks were placed as-hatched at a stocking density of 20 birds per square metre simulate commercial production systems. Birds were provided with feed and water *ad libitum* throughout the trial. Temperature and lighting adhered to the profiles as seen in appendices I and II, respectively. Tunnel ventilation was utilised, as was artificial lighting for light periods that extended beyond daylight hours. Vaccinations were administered through drinking water for Newcastle disease (on days 12 and 18 of the trial) and infectious bronchitis (on day 12 of the trial). Dead birds were collected twice daily, and their carcass weights recorded. Ethical approval was sanctioned by the ethics committee of the Faculty of Natural and Agricultural Sciences, University of Pretoria, with the reference number EC160331-010.

3.3 Experimental diets

Experimental diets were fed from days zero to 21, where after all birds were fed the same control diets. Grower was fed from days 22 till 27, Finisher was fed from days 28 till 30 and Post-finisher was fed from days 31 till 35. All feed was produced at a commercial feed mill (AFGRI Animal Feeds, Isando, South Africa). The ME and TLys concentrations as well as ME:TLys are provided in Table 3.1. Formulated raw material and nutrient compositions (%) of experimental diets are listed in Table 3.2 and Table 3.3, respectively. Analysed nutrient composition (%) of experimental diets may be viewed in Table 3.4.

Formulated and analysed total amino acid composition (%) of experimental diets is listed in Table 3.5 and Table 3.6, respectively.

Chemical analysis of feed samples was conducted at Labworld (Philafrica Feeds, Isando, South Africa). Moisture determination based on the AOAC official method 44 15-A (AOAC,1999) was conducted by comparing before and after weights of samples dried for 24 hours in an oven at 100°C. These values were used to calculate dry matter content by subtracting the moisture content from 100%. Protein analysis based on the AOAC official method 992.23 (AOAC, 2000), was conducted using a Gerhardt Dumatherm (AE Solutions, Centurion, South Africa) which determined the sample nitrogen content which was then multiplied by a factor of 6.25% to calculate the protein content. Crude fat analysis was based on the AOAC official method 954.02 (AOAC, 2000), whereby crude fat content was determined by heat extraction of the sample submerged in petroleum ether using a Gerhardt Soxhlet (AE Solutions, Centurion, South Africa). Crude fibre analysis was based on the AOAC official method 978.10 (AOAC, 2000), whereby the crude fibre content was determined using a Gerhardt Fibretherm programme (AE Solutions, Centurion, South Africa), which was followed by ashing of the sample. Ash content was determined based on the AOAC official method 942.05 (AOAC, 2000), by ashing samples 550°C for six hours. Calcium and phosphorus contents were determined by using a Gerhardt Skalar digestion process, (AE Solutions, Centurion, South Africa) with analysis based on the AOAC official method 976.06 (AOAC, 2000). Sodium and potassium contents were determined by absorption emission spectrometry based on an in-house method. This consisted of samples being ashed at 500°C for five hours before undergoing digestion with 32% hydrochloric acid on a heated digestion block until completely dry. Digested samples were then dissoluted with 1M hydrochloric acid and analysed on the Shimadzu AA-7000 (Shimadzu, Roodepoort, South Africa). Starch content was determined using spectrophotometric analysis on the ShimadzuUV-1800 (Shimadzu, Roodepoort, South Africa). with analysis based on the AOAC official method 996.11 (AOAC, 2000). Amino acids were analysed using high performance liquid chromatography (HPLC) at AMINOLab (Evonik Industries, Essen, Germany).

Table 3.1 Formulated metabolisable energy (ME) and total lysine (TLys) levels and TLys:ME of experimental diets that were fed to broilers from 0 to 21 days of age

TREATMENT COMBINATION	ME (MJ/kg)	TLys (%)	TLys:ME
1	11.30	1.3	0.12
2	12.13		0.11
3	12.97		0.10
4	11.30	1.4	0.12
5	12.13		0.12
6	12.97		0.11
7	11.30	1.6	0.14
8	12.13		0.13
9	12.97		0.12
10	11.30	1.7	0.15
11	12.13		0.14
12	12.97		0.13

Table 3.2 Formulated raw material composition (%) of experimental diets

	T 1	**T 2**	**T 3**	**T 4**	**T 5**	**T 6**	**T 7**	**T 8**	**T 9**	**T 10**	**T 11**	**T 12**
Yellow maize	55.02	57.69	54.97	51.53	53.38	52.62	40.66	44.98	40.62	39.92	43.88	39.98
Soya oilcake meal	28.70	32.50	32.65	32.40	33.50	31.05	29.25	28.55	29.25	28.05	27.30	28.50
Sunflower oilcake	5.00	0.00	0.00	5.00	3.85	0.00	5.00	5.00	5.00	5.00	5.00	5.00
Full fat soya	0.00	0.00	0.00	0.00	0.00	0.00	10.00	10.00	10.00	9.75	10.00	8.95
Fishmeal	0.00	0.00	0.00	0.00	0.00	3.60	1.00	1.00	1.00	4.55	4.65	4.65
Full fat maize germ	0.00	3.00	5.00	0.00	3.00	1.25	0.00	0.00	0.00	0.00	0.00	0.00
Wheat bran	5.00	2.55	0.00	5.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
White gluten	0.00	0.00	0.75	0.88	1.20	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soya oil (mixer)	0.00	0.00	2.35	0.00	0.78	1.50	0.50	1.30	2.48	0.00	0.83	2.29
Soya oil (coater)	0.00	0.00	0.00	0.00	0.00	1.50	0.00	0.00	2.48	0.00	0.00	2.29
Limestone	1.60	1.60	1.60	1.60	1.58	1.43	1.55	1.55	1.55	1.35	1.35	1.35
Mono di-calcium phosphate	1.10	1.13	1.15	1.03	1.08	0.73	0.95	0.95	0.95	0.50	0.48	0.48
Fine salt	0.26	0.27	0.27	0.25	0.25	0.19	0.23	0.22	0.23	0.14	0.13	0.14
Sodium bicarbonate	0.29	0.26	0.26	0.30	0.29	0.28	0.30	0.31	0.30	0.33	0.34	0.33
L-Lysine HCl 99% feed grade	0.35	0.29	0.30	0.38	0.37	0.27	0.41	0.42	0.41	0.35	0.36	0.35
DL-Methionine 99% feed grade	0.25	0.25	0.25	0.27	0.27	0.18	0.28	0.27	0.28	0.27	0.26	0.27
L-Threonine 98.5% feed grade	0.05	0.04	0.04	0.06	0.05	0.00	0.04	0.03	0.04	0.03	0.03	0.03
Choline chloride	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
PLT 1 Aviax + Olaquindox	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
AXTRA PHY 1000 TPT Broilers	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Sand (as filler)	1.97	0.00	0.00	0.90	0.00	0.00	4.42	0.00	0.00	4.35	0.00	0.00

Table 3.3 Formulated nutrient composition (%) of experimental diets

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**
Volume	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Moisture	10.85	10.95	10.55	11.00	10.77	10.66	10.19	10.61	10.28	10.22	10.61	10.30
Crude protein	20.80	20.90	21.02	22.80	22.78	24.66	26.63	26.68	26.63	28.14	28.29	28.11
Crude fat	2.86	4.21	7.29	2.83	4.88	6.60	4.74	5.67	9.10	4.52	5.52	8.89
Crude fibre	3.98	3.08	2.98	4.08	3.75	2.76	4.15	4.22	4.15	4.07	4.14	4.03
Ash	5.96	5.84	5.76	6.09	5.93	5.73	6.22	6.22	6.22	6.06	6.07	6.04
Calcium	1.05	1.05	1.05	1.05	1.05	1.06	1.06	1.06	1.06	1.05	1.05	1.05
Phosphorus (total)	0.67	0.64	0.63	0.68	0.65	0.62	0.67	0.68	0.67	0.66	0.67	0.66
Potassium	1.03	1.07	1.07	1.11	1.11	1.01	1.11	1.11	1.11	1.11	1.11	1.11
Sodium	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Metabolisable energy (MJ/kg)	11.56	12.44	13.28	11.59	12.48	13.25	11.57	12.39	13.18	11.57	12.40	13.20

Table 3.4 Analysed nutrient composition (%) of experimental diets

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**
Moisture	8.80	8.79	8.46	8.73	8.63	8.61	8.19	8.25	8.35	8.56	8.54	8.52
Dry matter content	91.20	91.21	91.54	91.27	91.37	91.39	91.81	91.75	91.65	91.44	91.46	91.48
Protein	21.09	21.90	21.64	23.06	23.26	25.10	26.79	27.54	26.61	29.20	29.51	28.57
Fat	3.28	4.89	7.62	3.43	5.67	6.57	4.89	5.59	9.29	5.06	5.16	8.64
Fibre	3.11	2.69	3.14	2.29	2.76	2.07	3.33	3.64	3.16	3.49	3.52	4.20
Ash	6.88	5.00	5.11	6.32	5.32	4.85	9.23	5.52	5.33	8.49	5.32	5.10
Calcium	0.86	0.86	0.82	0.93	0.95	0.82	1.02	0.93	0.86	0.86	0.86	0.88
Phosphate	0.65	0.64	0.66	0.59	0.64	0.62	0.66	0.62	0.65	0.54	0.64	0.64
Potassium	0.88	0.88	0.86	0.94	0.92	0.84	1.00	0.98	0.94	0.98	0.99	0.97
Sodium	0.19	0.20	0.20	0.22	0.20	0.23	0.22	0.20	0.23	0.26	0.23	0.22
Starch	37.15	38.21	35.53	37.83	34.78	34.12	28.20	30.82	27.61	27.53	28.99	27.77

Table 3.5 Formulated total amino acid composition (%) of experimental diets

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**
Lysine	1.31	1.31	1.31	1.44	1.43	1.44	1.63	1.63	1.63	1.72	1.72	1.71
Methionine	0.57	0.57	0.57	0.63	0.63	0.62	0.71	0.71	0.71	0.76	0.76	0.76
Methionine + Serine	0.92	0.92	0.92	1.00	1.00	1.01	1.14	1.14	1.14	1.19	1.20	1.19
Threonine	0.82	0.82	0.82	0.90	0.89	0.92	1.02	1.02	1.02	1.08	1.09	1.08
Tryptophan	0.23	0.23	0.23	0.26	0.25	0.26	0.29	0.29	0.29	0.30	0.30	0.30
Arginine	1.38	1.36	1.35	1.50	1.48	1.50	1.69	1.69	1.69	1.78	1.78	1.78
Isoleucine	0.85	0.87	0.88	0.94	0.95	1.03	1.13	1.12	1.13	1.19	1.19	1.19
Valine	0.97	0.98	0.98	1.07	1.06	1.16	1.25	1.25	1.25	1.32	1.33	1.32
Leucine	1.73	1.80	1.86	1.92	1.98	2.36	2.42	2.44	2.42	2.53	2.56	2.53
Histidine	0.56	0.57	0.57	0.61	0.61	0.65	0.69	0.69	0.69	0.73	0.73	0.73
Serine	1.01	1.04	1.05	1.11	1.12	1.22	1.32	1.32	1.32	1.37	1.38	1.37
Glycine	0.88	0.86	0.85	0.96	0.94	1.02	1.09	1.09	1.09	1.21	1.21	1.21
Glycine + Serine	1.89	1.89	1.90	2.07	2.06	2.24	2.41	2.41	2.41	2.58	2.59	2.58
Phenylalanine	1.00	1.02	1.04	1.11	1.12	1.24	1.34	1.34	1.34	1.39	1.40	1.39
Tyrosine	0.72	0.75	0.77	0.80	0.82	0.93	0.99	0.99	0.99	1.03	1.04	1.03
Alanine	1.04	1.06	1.09	1.14	1.16	1.40	1.41	1.43	1.41	1.52	1.54	1.52
Asparagine	2.04	2.08	2.09	2.25	2.26	2.36	2.61	2.60	2.61	2.75	2.75	2.74
Glutamine	3.69	3.69	3.73	4.07	4.06	4.39	4.81	4.82	4.81	4.98	5.00	4.97
Proline	1.21	1.25	1.27	1.32	1.34	1.54	1.58	1.60	1.58	1.64	1.67	1.64

Table 3.6 Analysed amino acid composition (%) of experimental diets

	T1	**T2**	**T3**	**T4**	**T5**	**T6**	**T7**	**T8**	**T9**	**T10**	**T11**	**T12**
Lysine	1.34	1.37	1.36	1.57	1.57	1.47	1.65	1.69	1.68	1.88	1.82	1.79
Methionine	0.49	0.54	0.53	0.61	0.58	0.56	0.64	0.65	0.64	0.72	0.72	0.72
Threonine	0.83	0.83	0.83	0.90	0.93	0.91	1.02	1.02	1.02	1.14	1.10	1.11
Tryptophan	0.27	0.26	0.27	0.28	0.29	0.29	0.31	0.31	0.32	0.35	0.34	0.34
Arginine	1.52	1.49	1.50	1.60	1.67	1.58	1.82	1.81	1.79	1.98	1.88	1.91
Isoleucine	0.90	0.89	0.90	0.98	1.00	1.04	1.15	1.12	1.12	1.27	1.22	1.25
Valine	1.02	1.00	1.02	1.09	1.12	1.18	1.28	1.26	1.25	1.39	1.35	1.38
Leucine	1.82	1.87	1.88	2.05	2.06	2.39	2.49	2.47	2.47	2.64	2.57	2.61
Histidine	0.57	0.57	0.58	0.61	0.63	0.63	0.69	0.68	0.67	0.74	0.71	0.72
Serine	1.09	1.09	1.11	1.18	1.21	1.25	1.37	1.38	1.36	1.47	1.40	1.42
Glycine	0.91	0.87	0.89	0.93	0.99	1.00	1.11	1.10	1.08	1.25	1.20	1.21
Phenylalanine	1.11	1.09	1.12	1.20	1.22	1.31	1.43	1.40	1.42	1.54	1.49	1.52
Alanine	1.08	1.10	1.12	1.19	1.21	1.41	1.44	1.44	1.43	1.57	1.54	1.55
Asparagine	2.19	2.20	2.20	2.38	2.43	2.41	2.71	2.68	2.66	2.93	2.81	2.85
Glutamine	4.03	3.94	3.98	4.28	4.42	4.58	5.10	5.06	5.04	5.40	5.16	5.24
Proline	1.24	1.27	1.27	1.37	1.34	1.49	1.58	1.59	1.59	1.71	1.60	1.67

3.4 Broiler performance measurements

At the outset of the trial, two feeders and one feed storage bin were allocated to each pen. These were clearly labelled with the corresponding pen number and their weights recorded, before being allocated 50 kg trial feed per pen. These empty feeder and bin weights were then subtracted from total feeder (with feed) and bin (with feed) weights to calculate the residual feed at each weighing interval (on days three, seven, 10, 14, 21, 28 and 35). By subtracting residual feed weight from initial feed weight, feed intake was calculated per pen. The same principle was applied to calculate feed intake for the Grower, Finisher and Post-finisher feeds in the carry-over period of the trial.

Bird counts were conducted at each weighing interval by manually transferring live birds into a crate (the weight of which was already tared on the scale). Dead birds were collected, counted and weighed daily with records being taken for each pen. These values were used to verify correct bird counts on weighing intervals. Crates containing all birds per pen were then weighed and this value was divided by the bird count to calculate the average bird weight per pen.

Using these measured values of average bird weight, feed intake and mortality rate, the following performance objectives were calculated based on the recommendations of Aviagen (2015):

BWG was calculated by subtracting the previous measured BW from the BW at the current weighing interval.

FCR was calculated using the formula $\frac{FI\ (kg)}{BWG\ (kg)}$

Liveability was calculated by subtracting mortality (%) from 100.

PEF was calculated using the formula $\frac{Liveability\ (\%) \times BW\ (kg)}{Age\ (d) \times FCR\ (kg\ feed\ per\ kg\ gain)} \times 100$

3.5 Carcass analysis

In an effort to eliminate the effect of sex on carcass composition differences, only male birds were evaluated in this trial. Following the weighing procedure on day 21, two birds were randomly selected from each pen. They were individually weighed and had neck tags inserted. These birds were then transported to an abattoir situated on the Hillcrest Experimental Farm (University of Pretoria, South Africa), where they were electrically stunned and exsanguinated by incision to the carotid artery and jugular veins. Carcasses were then de-feathered in an automated instrument that applied the principle of a tumbling motion which caused feathers to come loose and separate from the carcass. These carcasses were then manually eviscerated before being packaged and frozen. Once completely frozen, carcasses were minced using an electric mincer

and re-frozen before freeze drying (Nutrilab, University of Pretoria). Dried samples were then analysed for fat and protein content (Labworld, Philafrica Feeds, Isando, South Africa) with results reported as a percentage of sample weight.

Carcass moisture was determined using an in-house method of calculating the weight difference of samples before and after freeze drying. Carcass fat was analysed on AOAC official method 992.06 (AOAC, 2000), by first digesting samples with hydrochloric acid at high temperatures (acid hydrolysis) using a Gerhardt Hydrotherm (AE Solutions, Centurion, South Africa) and thereafter conducting crude fat extraction of the digested samples (heat extraction of the sample submerged in petroleum ether using a Gerhardt Soxhlet (AE Solutions, Centurion, South Africa). Protein analysis was conducted based on AOAC official method 992.23 (AOAC, 2000), using a Gerhardt Dumatherm (AE Solutions, Centurion, South Africa), which determined the sample nitrogen content which was then multiplied by a factor of 6.25% to calculate protein content.

3.6 Statistical analysis

The GLM model was applied to analyse data for average effects throughout the duration of the trial, with data having been treated as a randomised block design (Statistical Analysis Systems, 2016). Measurements recorded at days three, seven, 10, 14, 21, 28 and 35 were analysed using Repeated Measures Analysis of Variance with the GLM model (Statistical Analysis Systems, 2016). The Fischer's test (Samuels, 1989) was used to determine the significance of difference ($P < 0.05$) between calculated means and standard deviations. Equation of the linear model applied:

$$Y_{ijk} = \mu + L_i + T_j + B_k + LT_{ij} + e$$

Where Y is the variable of interest

μ is the overall population mean

L is the effect of the i^{th} level (ME)

T is the effect of the j^{th} treatment (TLys)

B is the effect of the k^{th} block

LT is effect of the i x j interaction (TLys × ME)

And e is the error associated with each Y

Chapter 4
Results

The following tables contain the main effects of treatment (TLys) and level (ME), as well as the interaction between treatment and level for the different dependent variables at different intervals in the trial. Blocks were not significant in any of the analysis and thus excluded in the result report.

4.1 Body weight

Results for body weight of broilers according to weighing intervals are shown below in Tables 4.1.1 to 4.1.8.

Table 4.1.1 The effect of varying ME:TLys on body weight (g) of broilers at 0 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	43.87 ($\pm$0.4154)^{12}	44.17 ($\pm$0.4154)	44.17 ($\pm$0.4154)	44.07 ($\pm$0.2398)
1,4	43.36 ($\pm$0.4856)^{2}	43.63 ($\pm$0.4154)	43.91 ($\pm$0.4154)	43.63 ($\pm$0.2541)
1,6	44.75 ($\pm$0.4154)^{1}	43.94 ($\pm$0.4154)	44.19 ($\pm$0.4856)	44.29 ($\pm$0.2541)
1,7	43.29 ($\pm$0.4154)^{2}	44.21 ($\pm$0.4154)	43.63 ($\pm$0.4154)	43.71 ($\pm$0.2398)
Mean	43.82 ($\pm$0.217)	43.99 ($\pm$0.2077)	43.97 ($\pm$0.217)	

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.1.2 The effect of varying ME:TLys on body weight (g) of broilers at 3 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	83.50 ($\pm$1.099)^{b2}	82.54 ($\pm$1.099)^{b2}	88.46 ($\pm$1.099)^{a}	84.83 ($\pm$0.6347)^{2}
1,4	88.23 ($\pm$1.285)^{1}	87.12 ($\pm$1.099)^{1}	88.86 ($\pm$1.099)	88.07 ($\pm$0.6723)^{1}
1,6	86.63 ($\pm$1.099)^{12}	88.84 ($\pm$1.099)^{1}	89.85 ($\pm$1.285)	88.44 ($\pm$0.6723)^{1}
1,7	89.54 ($\pm$1.099)^{a1}	86.08 ($\pm$1.099)^{b1}	87.54 ($\pm$1.099)^{ab}	87.72 ($\pm$0.6347)^{1}
Mean	86.97 ($\pm$0.5743)^{a}	86.15 ($\pm$0.5496)^{a}	88.68 ($\pm$0.5743)^{b}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.1.3 The effect of varying ME:TLys on body weight (g) of broilers at 7 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	162.3 (±3.081)^{b3}	155.1 (±3.081)^{b3}	184.3 (±3.081)^{a2}	167.2 (±1.779)^{2}
1,4	192.4 (±3.602)^{ab1}	185.5 (±3.081)^{b2}	197.2 (±3.081)^{a1}	191.7 (±1.884)^{1}
1,6	177.5 (±3.081)^{b2}	195.0 (±3.081)^{a1}	193.9 (±3.602)^{a12}	188.8 (±1.884)^{1}
1,7	187.1 (±3.081)^{1}	193.3 (±3.081)^{12}	195.0 (±3.081)^{1}	191.8 (±1.779)^{1}
Mean	179.8 (±1.610)^{b}	182.2 (±1.541)^{b}	192.6 (±1.610)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.1.4 The effect of varying ME:TLys on body weight (g) of broilers at 10 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	228.0 (±3.878)^{b3}	216.9 (±3.878)^{b3}	267.8 (±3.878)^{a2}	237.6 (±2.239)^{3}
1,4	273.6 (±4.534)^{b1}	268.1 (±3.878)^{b2}	286.5 (±3.878)^{a1}	276.1 (±2.372)^{12}
1,6	247.7 (±3.878)^{b2}	281.0 (±3.878)^{a1}	281.7 (±4.534)^{a1}	270.2 (±2.372)^{2}
1,7	277.7 (±3.878)^{1}	281.9 (±3.878)^{1}	284.1 (±3.878)^{1}	281.2 (±2.239)^{1}
Mean	256.7 (±2.026)^{b}	262.0 (±1.939)^{b}	280.0 (±2.026)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.1.5 The effect of varying ME:TLys on body weight (g) of broilers at 14 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	327.7 (±5.895)^{b3}	361.54 (±5.895)^{b3}	451.7 (±5.895)^{a2}	395.3 (±3.403)^{4}
1,4	459.6 (±6.891)^{b1}	451.0 (±5.895)^{b2}	485.8 (±5.895)^{a1}	465.5 (±3.605)^{2}
1,6	406.7 (±5.895)^{b2}	472.9 (±5.895)^{a1}	476.5 (±6.891)^{a1}	452.0 (±3.605)^{3}
1,7	475.0 (±5.895)^{1}	477.2 (±5.895)^{1}	484.3 (±5.895)^{1}	478.8 (±3.403)^{1}
Mean	428.5 (±3.080)^{c}	440.7 (±2.947)^{b}	474.6 (±3.080)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-4] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.1.6 The effect of varying ME:TLys on body weight (g) of broilers at 21 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	718.5 (±27.20)^{b2}	705.6 (±27.20)^{b2}	881.4 (±27.20)^{a}	768.5 (±15.70)^{2}
1,4	876.4 (±31.79)^{1}	900.3 (±27.20)^{1}	931.4 (±27.20)	902.7 (±16.63)^{1}
1,6	786.0 (±27.20)^{b23}	919.8 (±27.20)^{a1}	910.0 (±31.79)^{a}	871.9 (±16.63)^{1}
1,7	817.8 (±27.20)^{b12}	924.5 (±27.20)^{a1}	928.7 (±27.20)^{a}	890.3 (±15.70)^{1}
Mean	799.6 (±14.21)^{c}	862.6 (±13.60)^{b}	912.9 (±14.21)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.1.7 The effect of varying ME:TLys on body weight (g) of broilers at 28 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1267 (±18.44)^{b4}	1269 (±18.44)^{b2}	1466 (±18.44)^{a2}	1334 (±10.65)^{3}
1,4	1451 (±21.56)^{b2}	1481 (±18.44)^{ab1}	1530 (±18.44)^{a1}	1487 (±11.28)^{2}
1,6	1350 (±18.44)^{b3}	1531 (±18.44)^{a1}	1511 (±21.56)^{a12}	1464 (±11.28)^{2}
1,7	1511 (±18.44)^{1}	1531 (±18.44)^{1}	1547 (±18.44)^{1}	1530 (±10.65)^{1}
Mean	1395 (±9.635)^{c}	1453 (±9.221)^{b}	1514 (±9.635)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-4] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.1.8 The effect of varying ME:TLys on body weight (g) of broilers at 35 days of age ± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1846 (±26.60)^{b3}	1880 (±26.60)^{b2}	2058 (±26.60)^{a2}	1928 (±15.36)^{3}
1,4	2066 (±31.10)^{1}	2089 (±26.60)^{1}	2141 (±26.60)^{1}	2099 (±16.27)^{1}
1,6	1939 (±26.60)^{b2}	2096 (±26.60)^{a1}	2038 (±31.10)^{a2}	2025 (±16.27)^{2}
1,7	2109 (±26.60)^{1}	2135 (±26.60)^{1}	2135 (±26.60)^{1}	2127 (±15.36)^{1}
Mean	1990 (±13.90)^{c}	2050 (±13.30)^{b}	2093 (±13.90)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

No differences were observed between treatments for body weight (BW) at day zero, with the exception of TLys1.6 at ME 11.3, which was slightly heavier at the start of the trial. BW remained highest at ME 12.97 throughout the trial when broilers were fed the lowest TLys level (TLys1.3). Upon increasing TLys levels to TLys1.4, BW was highest at ME 12.97. This effect persisted from days three to 14, following which no differences were observed between ME levels. Improved BW was seen in response to increasing ME levels from ME 11.30 to ME 12.13 at both TLys1.6 and TLys1.7. Carryover effects resulted only from TLys1.6 with BW being highest at the termination of the trial (day 35). At ME 11.30, BW increased from TLys1.3 to TLys1.4 from days three to 21, and increased with each TLys level on days 28 and 35. Similarly, BW increased from TLys1.3 to TLys1.4 from days three to 35, and further increased from TLys1.4 to TLys1.6 from days 7 to 14 at ME 12.13. Carryover effects resulted only at ME 12.97, where BW increased from TLys1.3 to TLys1.4 from days seven to 14 and on days 28 and 35.

4.2 Body weight gain

Results for cumulative body weight gain according to weighing intervals are shown below in Tables 4.2.1 to 4.2.7.

Table 4.2.1 The effect of varying ME:TLys on body weight gain (g) of broilers at 3 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	13.21 (±0.3139)^{b2}	12.79 (±0.3139)^{b3}	14.77 (±0.3139)^{a}	13.59 (±0.1812)^{2}
1,4	14.95 (±0.3669)^{1}	14.50 (±0.3139)^{12}	14.99 (±0.3139)	14.81 (±0.1920)^{1}
1,6	13.96 (±0.3139)^{b2}	14.97 (±0.3139)^{a1}	15.22 (±0.3669)^{a}	14.71 (±0.1920)^{1}
1,7	15.52 (±0.3139)^{a1}	13.96 (±0.3139)^{b2}	14.64 (±0.3139)^{ab}	14.67 (±0.1812)^{1}
Mean	14.39 (±0.1640)^{b}	14.05 (±0.1569)^{b}	14.90 (±0.1640)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.2.2 The effect of varying ME:TLys on body weight gain (g) of broilers at 7 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	16.92 (±0.4156)^{b3}	15.84 (±0.4156)^{b3}	20.01 (±0.4156)^{a2}	17.59 (±0.2399)^{2}
1,4	21.30 (±0.4858)^{b1}	20.20 (±0.4156)^{b2}	21.89 (±0.4156)^{a1}	21.15 (±0.2542)^{1}
1,6	18.97 (±0.4156)^{b2}	21.58 (±0.4156)^{a1}	21.38 (±0.4858)^{a1}	20.64 (±0.2542)^{1}
1,7	20.54 (±0.4156)^{1}	21.29 (±0.4156)^{21}	21.63 (±0.4156)^{1}	21.15 (±0.2399)^{1}
Mean	19.43 (±0.2171)^{b}	19.74 (±0.2078)^{b}	21.23 (±0.2171)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.2.3 The effect of varying ME:TLys on body weight gain (g) of broilers at 10 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	21.90 (±0.4976)^{b4}	20.61 (±0.4976)^{b3}	27.83 (±0.4976)^{a2}	23.45 (±0.2873)^{4}
1,4	27.05 (±0.5817)^{b2}	27.53 (±0.4976)^{b2}	29.78 (±0.4976)^{a1}	28.12 (±0.3043)^{2}
1,6	23.39 (±0.4976)^{b3}	28.68 (±0.4976)^{a12}	29.29 (±0.5817)^{a12}	27.12 (±0.3043)^{3}
1,7	30.21 (±0.4976)^{1}	29.56 (±0.4976)^{1}	29.68 (±0.4976)^{1}	29.82 (±0.2399)^{1}
Mean	25.64 (±0.2599)^{c}	26.60 (±0.2488)^{b}	29.14 (±0.2599)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-4] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.2.4 The effect of varying ME:TLys on body weight gain (g) of broilers at 14 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	30.06 (±0.4986)^{b4}	29.49 (±0.4986)^{b3}	38.21 (±0.4986)^{a2}	32.59 (±0.2879)^{4}
1,4	36.18 (±0.5829)^{b2}	37.93 (±0.4986)^{b2}	41.24 (±0.4986)^{a1}	39.11 (±0.3849)^{2}
1,6	32.74 (±0.4986)^{b3}	39.7 (±0.4986)^{a1}	40.37 (±0.5829)^{a1}	37.60 (±0.3849)^{3}
1,7	41.13 (±0.4986)^{1}	40.56 (±0.4986)^{1}	41.32 (±0.4986)^{1}	41.00 (±0.2879)^{1}
Mean	35.52 (±0.2605)^{b}	32.92 (±0.2493)^{c}	40.28 (±0.2605)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-4] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.2.5 The effect of varying ME:TLys on body weight gain (g) of broilers at 21 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	49.39 (±3.519)^{b}	49.15 (±3.519)^{b2}	61.39 (±3.519)^{a}	51.31 (±2.032)^{2}
1,4	59.54 (±4.114)	64.19 (±3.519)^{1}	63.66 (±3.519)	62.46 (±2.153)^{1}
1,6	54.19 (±3.519)	63.85 (±3.519)^{1}	61.93 (±4.114)	59.99 (±2.153)^{1}
1,7	48.98 (±3.519)^{b}	63.90 (±3.519)^{a1}	63.49 (±3.519)^{a}	58.79 (±2.032)^{12}
Mean	53.02 (±1.839)^{b}	60.27 (±1.760)^{a}	62.62 (±1.839)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.2.6 The effect of varying ME:TLys on body weight gain (g) of broilers at 28 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	78.41 (±4.188)^{2}	80.47 (±4.188)	83.5 (±4.188)	80.79 (±2.418)^{2}
1,4	82.07 (±4.896)^{2}	82.92 (±4.188)	85.56 (±4.188)	83.52 (±2.562)^{2}
1,6	80.63 (±4.188)^{2}	87.35 (±4.188)	85.84 (±4.896)	84.60 (±2.562)^{12}
1,7	99.10 (±4.188)^{a1}	86.59 (±4.188)^{b}	88.35 (±4.188)^{ab}	91.34 (±2.418)^{1}
Mean	85.05 (±2.188)	84.33 (±2.094)	85.81 (±2.188)	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.2.7 The effect of varying ME:TLys on body weight gain (g) of broilers at 35 days of age ± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	82.71 (±2.311)	87.35 (±2.311)	84.54 (±2.311)^{1}	84.86 (±1.334)^{1}
1,4	87.90 (±2.702)	86.86 (±2.311)	87.22 (±2.311)^{1}	87.33 (±1.414)^{1}
1,6	84.04 (±2.311)^{a}	80.75 (±2.311)^{ab}	75.37 (±2.702)^{b2}	80.07 (±1.414)^{2}
1,7	85.42 (±2.311)	86.35 (±2.311)	84.01 (±2.311)^{1}	85.26 (±1.334)^{1}
Mean	85.03 (±1.207)	85.33 (±1.156)	82.78 (±1.207)	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Body weight gain (BWG) in broilers was highest at ME 12.97 at TLys1.3 and TLys1.4 throughout the experimental feeding phase (days 0 to 21). At TLys1.6, BWG increased from ME 11.30 to ME 12.13 from days three to 14. This effect persisted up to day 35. A similar effect was observed at TLys1.7, where BWG increased from ME 11.30 to ME 12.13, with a carryover effect until day 28. At ME 11.30, BWG increased with increasing TLys levels from TLys1.3 to TLys1.4 from days three to 14, and from TLys1.4 to TLys1.7 on days 10 and 14. On day 28, BWG was highest at TLys1.7. This trend continued at ME 12.13, where BWG increased with increasing TLys levels within the experimental feeding phase. At ME 12.97, BWG increased from TLys1.3 to TLys1.4 from days seven to 14 and was lowest at TLys1.6 on day 35.

4.3 Feed intake

Results for cumulative feed intake according to weighing intervals are shown below in Tables 4.3.1 to 4.3.7.

Table 4.3.1 The effect of varying ME:TLys on feed intake (g) of broilers from 0 to 3 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	41.71 (±1.394)	41.92 (±1.394)^{1}	40.29 (±1.394)	41.31 (±0.8046)
1,4	41.08 (±1.629)	37.88 (±1.394)^{2}	39.02 (±1.394)	39.38 (±0.8523)
1,6	41.21 (±1.394)^{a}	39.17 (±1.394)^{a12}	37.43 (±1.629)^{b}	39.27 (±0.8523)
1,7	40.00 (±1.394)	40.29 (±1.394)^{12}	37.67 (±1.394)	39.32 (±0.8046)
Mean	41.00 (±0.7280)^{a}	39.81 (±0.6968)^{ab}	38.60 (±0.7280)^{b}	

^{a,b} Means within the same row with no common superscript differ significantly (P < 0.05)

^{1,2} Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.3.2 The effect of varying ME:TLys on feed intake (g) of broilers from 0 to 7 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	162.1 (±4.855)	155.4 (±4.855)^{2}	160.9 (±4.855)	159.5 (±2.803)
1,4	173.0 (±5.676)	159.3 (±4.855)^{2}	167.6 (±4.855)	166.6 (±2.970)
1,6	162.3 (±4.855)^{b}	177.9 (±4.855)^{a1}	153.0 (±5.676)^{b}	164.4 (±2.970)
1,7	164.6 (±4.855)	165.1 (±4.855)^{12}	161.5 (±4.855)	163.8 (±2.803)
Mean	165.5 (±2.536)	164.4 (±2.428)	160.8 (±2.536)	

^{a,b} Means within the same row with no common superscript differ significantly (P < 0.05)

^{1,2} Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.3.3 The effect of varying ME:TLys on feed intake (g) of broilers from 0 to 10 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	268.7 (±5.117)^{a2}	251.7 (±5.117)^{b2}	275.5 (±5.117)^{a12}	265.3 (±2.954)^{2}
1,4	290.3 (±5.982)^{a1}	272.8 (±5.117)^{b1}	289.0 (±5.117)^{a1}	284.1 (±3.130)^{1}
1,6	267.2 (±5.117)^{2}	277.6 (±5.117)^{1}	268.8 (±5.982)^{2}	271.2 (±3.130)^{2}
1,7	290.8 (±5.117)^{1}	284.8 (±5.117)^{1}	279.8 (±5.117)^{12}	285.1 (±2.954)^{1}
Mean	279.2 (±2.673)	271.7 (±2.558)	278.3 (±2.673)	

^{a,b} Means within the same row with no common superscript differ significantly (P < 0.05)

^{1,2} Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.3.4 The effect of varying ME:TLys on feed intake (g) of broilers from 0 to 14 days of age (± standard error of the mean)

Metabolisable Energy (MJ/kg)				
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	486.2 (±10.08)^{a2}	466.1 (±10.08)^{a2}	521.8 (±10.08)^{b}	491.3 (±5.818)^{3}
1,4	546.0 (±11.78)^{1}	521.8 (±10.08)^{1}	549.5 (±10.08)	539.1 (±6.163)^{1}
1,6	494.5 (±10.08)^{a2}	526.7 (±10.08)^{b1}	518.4 (±11.78)^{ab}	513.2 (±6.163)^{2}
1,7	572.0 (±10.08)^{a1}	539.2 (±10.08)^{b1}	528.0 (±10.08)^{b}	546.4 (±5.818)^{1}
Mean	542.7 (±5.264)^{ab}	513.4 (±5.038)^{a}	529.4 (±5.264)^{b}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.3.5 The effect of varying ME:TLys on feed intake (g) of broilers from 0 to 21 days of age (± standard error of the mean)

Metabolisable Energy (MJ/kg)				
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1052 (±20.26)^{b3}	1027 (±20.26)^{b3}	1178 (±20.26)^{a}	1086 (±11.70)^{3}
1,4	1209 (±23.68)^{2}	1173 (±20.26)^{2}	1229 (±20.26)	1204 (±12.39)^{1}
1,6	1107 (±20.26)^{b3}	1194 (±20.26)^{a12}	1190 (±23.68)^{a}	1163 (±12.39)^{2}
1,7	1279 (±20.26)^{a1}	1235 (±20.26)^{ab1}	1181 (±20.26)^{b}	1231 (±11.70)^{1}
Mean	1162 (±10.58)^{b}	1157 (±10.13)^{b}	1194 (±10.58)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.3.6 The effect of varying ME:TLys on feed intake (g) of broilers from 0 to 28 days of age (± standard error of the mean)

Metabolisable Energy (MJ/kg)				
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1866 (±29.77)^{b3}	1862 (±29.77)^{b3}	2062 (±29.77)^{a}	1930 (±17.19)^{4}
1,4	2097 (±34.80)^{2}	2077 (±29.77)^{2}	2161 (±29.77)	2112 (±18.21)^{2}
1,6	1964 (±29.77)^{b3}	2101 (±29.77)^{a12}	2106 (±34.80)^{a}	2057 (±18.21)^{3}
1,7	2231 (±29.77)^{a1}	2172 (±29.77)^{ab1}	2139 (±29.77)^{b}	2181 (±17.19)^{1}
Mean	2040 (±15.55)^{b}	2053 (±14.88)^{b}	2117 (±15.55)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-4] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.3.7 The effect of varying ME:TLys on feed intake (g) of broilers from 0 to 35 days of age ± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	2902 $(\pm 45.83)^{b2}$	2912 $(\pm 45.83)^{b2}$	3149 $(\pm 45.83)^{a2}$	2988 $(\pm 26.46)^{3}$
1,4	3234 $(\pm 53.58)^{1}$	3197 $(\pm 45.83)^{1}$	3298 $(\pm 45.83)^{1}$	3243 $(\pm 28.03)^{1}$
1,6	3017 $(\pm 45.83)^{b2}$	3181 $(\pm 45.83)^{a1}$	3194 $(\pm 53.58)^{a12}$	3131 $(\pm 28.03)^{2}$
1,7	3377 $(\pm 45.83)^{1}$	3310 $(\pm 45.83)^{1}$	3274 $(\pm 45.83)^{12}$	3321 $(\pm 26.46)^{1}$
Mean	3133 $(\pm 23.94)^{b}$	3150 $(\pm 22.91)^{b}$	3229 $(\pm 23.94)^{a}$	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Feed intake (FI) of broilers was lowest at high ME levels (ME 12.13 and ME 12.97) at TLys1.3 and TLys1.4 from days zero to 14. The highest FI observed at TLys1.4 resulted from ME 12.97, with a persistent carryover effect. At TLys1.6, FI increased from ME 11.30 to ME 12.13 from day fourteen to 35. In contrast, FI decreased with increasing ME levels at TLys1.7. At ME 11.30, FI increased from TLys1.3 to TLys1.4 from days 10 to 35, with a further increase from TLys1.4 to TLys1.7 on days 21 and 28. Similarly, at ME 12.13; FI increased from TLys1.3 to TLys1.4 from days 10 to 35, with a further increase from TLys1.4 to TLys1.7 on days 21 and 28. This effect continued at ME 12.97; where FI increased from TLys1.4 to TLys1.6 on day 10 and from TLys1.3 to TLys1.4 on day 35.

4.4 Feed conversion ratio

Results for cumulative feed conversion ratio according to weighing intervals are shown below from Tables 4.4.1 to 4.4.7.

Table 4.4.1 The effect of varying ME:TLys on the feed conversion ratio (kg FI/ kg BWG) of broilers from 0 to 3 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1.053 $(\pm 0.0391)^{a1}$	1.093 $(\pm 0.0391)^{a1}$	0.9100 $(\pm 0.0391)^{b}$	1.018 $(\pm 0.0178)^{1}$
1,4	0.9176 $(\pm 0.0361)^{23}$	0.8710 $(\pm 0.0391)^{2}$	0.8683 (± 0.0391)	0.8856 $(\pm 0.0189)^{2}$
1,6	0.9840 $(\pm 0.0391)^{a12}$	0.8728 $(\pm 0.0391)^{b2}$	0.8206 $(\pm 0.0361)^{b}$	0.8925 $(\pm 0.0189)^{2}$
1,7	0.8655 $(\pm 0.0391)^{b3}$	0.9655 $(\pm 0.0391)^{a1}$	0.8578 $(\pm 0.0391)^{b}$	0.8963 $(\pm 0.0178)^{2}$
Mean	0.9594 $(\pm 0.0162)^{a}$	0.9504 $(\pm 0.0155)^{a}$	0.8642 $(\pm 0.0162)^{b}$	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.4.2 The effect of varying ME:TLys on the feed conversion ratio (kg FI/ kg BWG) of broilers from 0 to 7 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1.371 (±0.0288)^{a1}	1.402 (±0.0288)^{a1}	1.151 (±0.0288)^{b1}	1.308 (±0.0166)^{1}
1,4	1.162 (±0.0337)^{2}	1.123 (±0.0288)^{2}	1.093 (±0.0288)^{12}	1.126 (±0.0176)^{2}
1,6	1.233 (±0.0288)^{a2}	1.176 (±0.0288)^{a2}	1.022 (±0.0337)^{b2}	1.140 (±0.0176)^{2}
1,7	1.146 (±0.0288)^{2}	1.109 (±0.0288)^{2}	1.066 (±0.0288)^{2}	1.107 (±0.0166)^{2}
Mean	1.225 (±0.0150)^{a}	1.203 (±0.0144)^{a}	1.083 (±0.0150)^{b}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.4.3 The effect of varying ME:TLys on the feed conversion ratio (kg FI/ kg BWG) of broilers from 0 to 10 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1.461 (±0.0175)^{a1}	1.459 (±0.0175)^{a1}	1.234 (±0.0175)^{b1}	1.385 (±0.0101)^{1}
1,4	1.263 (±0.0204)^{a23}	1.216 (±0.0175)^{ab2}	1.192 (±0.0175)^{b12}	1.223 (±0.0107)^{2}
1,6	1.317 (±0.0175)^{a2}	1.171 (±0.0175)^{ab2}	1.131 (±0.0204)^{b3}	1.206 (±0.0107)^{2}
1,7	1.241 (±0.0175)^{a3}	1.199 (±0.0175)^{ab2}	1.163 (±0.0175)^{b23}	1.201 (±0.0101)^{2}
Mean	1.320 (±0.0091)^{a}	1.261 (±0.0087)^{b}	1.180 (±0.0091)^{c}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.4.4 The effect of varying ME:TLys on the feed conversion ratio (kg FI/ kg BWG) of broilers from 0 to 14 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1.480 (±0.0188)^{a1}	1.471 (±0.0188)^{a1}	1.281 (±0.0188)^{b1}	1.410 (±0.0109)^{1}
1,4	1.313 (±0.0220)^{a2}	1.281 (±0.0188)^{ab2}	1.244 (±0.0188)^{b1}	1.279 (±0.0115)^{2}
1,6	1.367 (±0.0188)^{a2}	1.228 (±0.0188)^{b2}	1.199 (±0.0220)^{b2}	1.264 (±0.0115)^{2}
1,7	1.325 (±0.0188)^{a2}	1.246 (±0.0188)^{ab2}	1.198 (±0.0188)^{b2}	1.256 (±0.0109)^{2}
Mean	1.371 (±0.0098)^{a}	1.306 (±0.0094)^{b}	1.230 (±0.0098)^{c}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.4.5 The effect of varying ME:TLys on the feed conversion ratio (kg FI/ kg BWG) of broilers from 0 to 21 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1.561 (±0.0638)^{12}	1.554 (±0.0638)^{1}	1.406 (±0.0638)	1.507 (±0.0368)
1,4	1.449 (±0.0746)^{2}	1.370 (±0.0638)^{2}	1.385 (±0.0638)	1.401 (±0.0390)
1,6	1.493 (±0.0638)^{2}	1.363 (±0.0638)^{2}	1.371 (±0.0746)	1.409 (±0.0390)
1,7	1.715 (±0.0638)^{a1}	1.403 (±0.0638)^{b12}	1.334 (±0.0638)^{b}	1.484 (±0.0368)
Mean	1.554 (±0.0333)^{a}	1.422 (±0.0319)^{b}	1.374 (±0.0333)^{b}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.4.6 The effect of varying ME:TLys on the feed conversion ratio (kg FI/ kg BWG) of broilers from 0 to 28 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1.526 (±0.0133)^{a}	1.521 (±0.0133)^{a1}	1.450 (±0.0133)^{b}	1.499 (±0.0077)^{1}
1,4	1.490 (±0.0156)^{a}	1.446 (±0.0133)^{b23}	1.454 (±0.0133)^{ab}	1.463 (±0.0081)^{2}
1,6	1.505 (±0.0133)^{a}	1.413 (±0.0133)^{b3}	1.435 (±0.0156)^{b}	1.451 (±0.0081)^{2}
1,7	1.520 (±0.0133)^{a}	1.463 (±0.0133)^{b2}	1.423 (±0.0133)^{c}	1.468 (±0.0077)^{2}
Mean	1.150 (±0.0070)^{c}	1.461 (±0.0067)^{b}	1.440 (±0.0070)^{b}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.4.7 The effect of varying ME:TLys on the feed conversion ratio (kg FI/ kg BWG) of broilers from 0 to 35 days of age ± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	1.610 (±0.0120)^{a12}	1.586 (±0.0120)^{ab1}	1.563 (±0.0120)^{b2}	1.587 (±0.0069)
1,4	1.598 (±0.0140)^{12}	1.563 (±0.0120)^{12}	1.573 (±0.0120)^{12}	1.578 (±0.0073)
1,6	1.593 (±0.0120)^{a2}	1.550 (±0.0120)^{b2}	1.602 (±0.0140)^{a1}	1.581 (±0.0073)
1,7	1.635 (±0.0120)^{a1}	1.584 (±0.0120)^{b12}	1.565 (±0.0120)^{b12}	1.594 (±0.0069)
Mean	1.609 (±0.0063)^{a}	1.571 (±0.0060)^{b}	1.576 (±0.0063)^{b}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

The calculated feed conversion ratio (FCR) of broilers was lowest at ME 12.13 and ME 12.97 at TLys1.3, with carryover effects observed at the termination of the trial. Similar results were observed at TLys1.4, with effects continuing till day twenty-eight of the trial. At TLys1.6, FCR was again favoured by the higher ME levels. FCR was lowest at ME 12.13 as a carryover effect at the termination of the trial. This trend persisted at TLys1.7, with FCR decreasing with increasing ME levels as a carryover effect. Carryover improvements in FCR were observed at all ME levels when TLys was increased up to TLys1.6, while an increase in TLys from TLys1.3 to TLys1.4 decreased FCR mostly within the experimental feeding phase.

4.5 Performance efficiency factor

Results for performance efficiency factor according to weighing intervals are shown below in Tables 4.5.1 to 4.5.7.

Table 4.5.1 The effect of varying ME:TLys on the performance efficiency of broilers at 3 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	265.6 (±11.39)^{b3}	252.6 (±11.39)^{b3}	325.6 (±11.39)^{a2}	281.3 (±6.573)^{2}
1,4	322.18 (±13.31)^{12}	333.7 (±11.39)^{1}	343.1 (±11.39)^{12}	333.0 (±6.964)^{1}
1,6	295.2 (±11.39)^{b23}	339.7 (±11.39)^{a1}	365.3 (±13.31)^{a1}	333.4 (±6.964)^{1}
1,7	346.3 (±11.39)^{a1}	299.6 (±11.39)^{b2}	341.0 (±11.39)^{a12}	328.9 (±6.573)^{1}
Mean	307.3 (±5.948)^{b}	306.4 (±5.639)^{b}	341.7 (±5.948)^{ab}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.5.2 The effect of varying ME:TLys on the performance efficiency of broilers at 7 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	169.0 (±7.202)^{b3}	158.1 (±7.202)^{b2}	229.7 (±7.202)^{a2}	185.6 (±4.158)^{2}
1,4	236.6 (±8.419)^{ab1}	235.2 (±7.202)^{b1}	258.0 (±7.202)^{a1}	243.2 (±4.405)^{1}
1,6	207.7 (±7.202)^{c2}	238.8 (±7.202)^{b1}	268.0 (±8.419)^{a1}	238.1 (±4.405)^{1}
1,7	233.9 (±7.202)^{b1}	249.5 (±7.202)^{ab1}	261.4 (±7.202)^{a1}	248.3 (±4.158)^{1}
Mean	211.8 (±3.762)^{b}	220.4 (±3.601)^{b}	254.3 (±3.762)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.5.3 The effect of varying ME:TLys on the performance efficiency of broilers at 10 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	156.0 (±4.496)^{b3}	148.9 (±4.496)^{b2}	217.9 (±4.496)^{a2}	174.3 (±2.596)^{3}
1,4	216.7 (±5.257)^{b1}	219.8 (±4.496)^{b1}	240.7 (±4.496)^{a1}	225.7 (±2.750)^{2}
1,6	188.2 (±4.496)^{c2}	239.1 (±4.496)^{b1}	246.8 (±5.257)^{a1}	224.7 (±2.750)^{2}
1,7	224.4 (±4.496)^{b1}	235.6 (±4.496)^{ab1}	244.4 (±4.496)^{a1}	234.8 (±2.596)^{1}
Mean	196.3 (±2.349)^{c}	210.8 (±2.348)^{b}	237.4 (±2.349)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.5.4 The effect of varying ME:TLys on the performance efficiency of broilers at 14 days of age (± standard error of the mean)

Metabolisable Energy (MJ/kg)				
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	179.6 (±4.695)^{b3}	175.9 (±4.695)^{b3}	252.4 (±4.695)^{a2}	202.6 (±2.711)^{3}
1,4	250.0 (±5.489)^{b1}	250.5 (±4.695)^{b2}	278.1 (±4.695)^{a1}	259.5 (±2.872)^{2}
1,6	212.7 (±4.695)^{b2}	272.9 (±4.695)^{a1}	280.9 (±5.489)^{a1}	255.5 (±2.872)^{2}
1,7	257.1 (±4.695)^{c1}	273.8 (±4.695)^{b1}	287.6 (±4.695)^{a1}	272.8 (±2.711)^{3}
Mean	224.9 (±2.453)^{c}	243.3 (±2.348)^{b}	274.7 (±2.453)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.5.5 The effect of varying ME:TLys on the performance efficiency of broilers at 21 days of age (± standard error of the mean)

Metabolisable Energy (MJ/kg)				
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	217.6 (±13.57)^{b2}	215.6 (±13.57)^{b2}	298.8 (±13.57)^{a}	244.0 (±7.833)^{2}
1,4	283.2 (±15.56)^{1}	310.8 (±13.57)^{1}	319.0 (±13.57)	304.3 (±7.088)^{1}
1,6	249.8 (±13.57)^{b12}	316.1 (±13.57)^{a1}	200.6 (±15.56)^{a}	288.8 (±7.088)^{1}
1,7	239.7 (±13.57)^{b2}	314.1 (±13.57)^{a1}	330.3 (±13.57)^{a}	294.7 (±7.833)^{1}
Mean	247.6 (±7.088)^{a}	289.1 (±6.784)^{b}	312.2 (±7.088)^{c}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.5.6 The effect of varying ME:TLys on the performance efficiency of broilers at 28 days of age (± standard error of the mean)

Metabolisable Energy (MJ/kg)				
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	289.4 (±6.376)^{b3}	294.4 (±6.376)^{b2}	356.7 (±6.376)^{a2}	313.5 (±3.681)^{3}
1,4	337.2 (±7.454)^{b1}	358.2 (±6.376)^{a1}	371.2 (±6.376)^{a12}	355.5 (±3.900)^{1}
1,6	308.8 (±6.376)^{b2}	367.8 (±6.376)^{a1}	352.2 (±7.454)^{a2}	342.9 (±3.900)^{2}
1,7	345.1 (±6.376)^{b1}	367.9 (±6.376)^{a1}	380.4 (±6.376)^{a1}	364.5 (±3.681)^{1}
Mean	320.1 (±3.331)^{c}	347.1 (±3.188)^{b}	365.1 (±3.331)^{a}	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Table 4.5.7 The effect of varying ME:TLys on the performance efficiency of broilers at 35 days of age ± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11,3**	**12,13**	**12,97**	**Mean**
1,3	308.6 (±8.862)^{b2}	318.8 (±8.862)^{b2}	352.4 (±8.862)^{a1}	326.6 (±5.117)^{2}
1,4	348.9 (±10.36)^{1}	356.4 (±8.862)^{1}	366.3 (±8.862)^{1}	357.2 (±5.420)^{1}
1,6	317. 1 (±8.862)^{b2}	352.7 (±8.862)^{a1}	317.7 (±10.36)^{b2}	329.2 (±5.420)^{2}
1,7	350.3 (±8.862)^{1}	356.6 (±8.862)^{1}	355.8 (±8.862)^{1}	354.2 (±5.117)^{1}
Mean	331.2 (±4.630)^{b}	346.1 (±4.431)^{a}	348.0 (±4.630)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1,2] Means within the same column with no common superscript differ significantly (P < 0.05)

The performance efficiency factor (PEF) of broilers was calculated using BW, cumulative mortality rate and cumulative FI. Across all ME levels, PEF increased in response to increasing TLys levels. At TLys1.3, PEF was highest at ME 12.97. Carryover effects observed for TLys1.6 and TLys1.7 proved that optimal PEF was achieved at ME 12.13. Additional carryover improvements in PEF resulted from increasing TLys levels from TLys1.3 to TLys1.4 at ME 11.30 and ME 12.13. At ME 12.97, PEF increased from TLys1.3 to TLys1.4 from day three to 14 and from TLys1.3 to TLys1.7 on day twenty-eight.

4.6 Carcass composition

Results for carcass analysis according to parameter analysed are shown below in Tables 4.6.1 to 4.6.7.

Table 4.6.1 The **e**ffect of varying ME: TLys ratios on carcass moisture (as % carcass weight) of broilers at 21 days of age (± standard error of the mean)

	Metabolisable Energy (MJ/kg)			
Total Lysine (g/kg)	**11.3**	**12.13**	**12.97**	**Mean**
1.3	68.99 (±0.5995)^{1}	67.40 (±0.5995)^{2}	68.10 (±0.5995)^{2}	67.84 (±0.3461)^{3}
1.4	68.62 (±0.7008)^{b2}	70.74 (±0.5995)^{a1}	71.23 (±0.5995)^{a1}	70.44 (±0.3666)^{2}
1.6	68.99 (±0.5995)^{b2}	71.52 (±0.5995)^{a1}	71.97 (±0.7008)^{a1}	71.02 (±0.3666)^{12}
1.7	73.00 (±0.5995)^{b1}	70.60 (±0.5995)^{a1}	71.23 (±0.5995)^{a1}	71.61 (±0.3461)^{1}
Mean	69.66 (±0.3132)^{b}	70.07 (±0.2997)^{b}	70.97(±0.3132)^{a}	

[a,b] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

ME 11.3 yielded the lowest carcass moisture at TLys1.4, TLys1.6 and TLys1.7. At ME 11.3, carcass moisture was highest at TLys1.7 and lowest at ME 12.13 and ME 12.97.

Table 4.6.2 The **e**ffect of varying ME: TLys ratios on carcass fat (as % carcass weight) of broilers at 21 days of age (± standard error of the mean)

	Metabolisable Energy			
Total Lysine	**11.3**	**12.13**	**12.97**	**Mean**
1.3	$29.78\ (\pm 1.147)^{b1}$	$36.78\ (\pm 1.147)^{a1}$	$25.22\ (\pm 1.147)^{c1}$	$30.59\ (\pm 0.6623)^{1}$
1.4	$25.29\ (\pm 1.341)^{2}$	$24.91\ (\pm 1.147)^{2}$	$23.47\ (\pm 1.147)^{12}$	$24.56\ (\pm 0.7016)^{2}$
1.6	$25.47\ (\pm 1.147)^{a2}$	$19.92\ (\pm 1.147)^{b3}$	$19.71\ (\pm 1.34)^{b3}$	$21.70\ (\pm 0.7016)^{3}$
1.7	$18.75\ (\pm 1.147)^{3}$	$20.07\ (\pm 1.147)^{3}$	$20.75\ (\pm 1.147)^{23}$	$19.85\ (\pm 0.6623)^{3}$
Mean	$24.82\ (\pm 0.5992)^{a}$	$25.42\ (\pm 0.5735)^{a}$	$22.29\ (\pm 0.5992)^{b}$	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-3] Means within the same column with no common superscript differ significantly (P < 0.05)

Fat content increased with an increase in ME levels from ME 11.3 to ME 12.13 at TLys1.3. In contrast, carcass fat was lowest at ME 12.97. At TLys1.6, carcass fat was highest at ME 11.30. Carcass fat decreased with increasing TLys levels across all ME levels.

Table 4.6.3 The **e**ffect of varying ME: TLys ratios on carcass protein (as % carcass weight) of broilers at 21 days of age (± standard error of the mean)

	Metabolisable Energy			
Total Lysine	**11.3**	**12.13**	**12.97**	**Mean**
1.3	$52.95\ (\pm 0.8914)^{c3}$	$46.91\ (\pm 0.8914)^{b3}$	$57.06\ (\pm 0.8914)^{a3}$	$52.31\ (\pm 0.5146)^{4}$
1.4	$56.64\ (\pm 1.042)^{2}$	$56.82\ (\pm 0.8914)^{2}$	$58.86\ (\pm 0.8914)^{23}$	$57.44\ (\pm 0.5452)^{3}$
1.6	$55.20\ (\pm 0.8914)^{b23}$	$61.80\ (\pm 0.8914)^{a1}$	$62.28\ (\pm 1.042)^{a1}$	$59.76\ (\pm 0.5452)^{2}$
1.7	$64.17\ (\pm 0.8914)^{a1}$	$60.72\ (\pm 0.8914)^{b1}$	$60.31\ (\pm 0.8914)^{b12}$	$61.73\ (\pm 0.5146)^{1}$
Mean	$57.24\ (\pm 0.4657)^{b}$	$56.56\ (\pm 0.4457)^{b}$	$59.63\ (\pm 0.4657)^{a}$	

[a-c] Means within the same row with no common superscript differ significantly (P < 0.05)

[1-4] Means within the same column with no common superscript differ significantly (P < 0.05)

An increase in carcass crude protein was observed with ME levels increasing from ME 11.3 to ME 12.97 at TLys1.3. Carcass protein also increased with increasing TLys levels across all ME levels. At both TLys1.6 and TLys1.7, carcass protein was lowest at ME 11.3.

4.7 Mortality

No significant differences were observed between treatments for mortality. Cumulative mortality averaged at 7.21% at the end of the trial.

Chapter 5
Discussion

5.1 Body weight and body weight gain

Body weight on day three was promoted by the combination of TLys1.3 and ME 12.97, which changed to TLys1.6 and ME 12.13 from days seven to 14. By the end of the treatment phase (day 21), optimal BW resulted from TLys1.4 with no significant difference between ME levels. This effect persisted throughout the carryover period until slaughter at day 35. For the greater extent of the trial, no significant differences were observed between ME levels at TLys1.7. BW at ME 12.13 and 12.97 did not respond to an increase beyond TLys1.4, with the exception of BW at ME 11.30 increasing at TLys1.7. This is consistent with findings of Madsen *et al.* (2010), who reported that increasing BP levels increased BW, more so at low ME levels. Response up to the highest TLys level was observed only at the ME 11.30. At TLys1.4, no response was elicited by differences in ME levels. At TLys1.6, BW improved with an increase from ME 11.30 to ME 12.13 but not at ME 12.97. At TLys1.3, BW increased from ME 11.30 to ME 12.97.

In contrast to the findings of this trial, no response was seen between ME levels at the lowest dLys level when examined by Chang *et al.* (2015). BW did not increase with increasing ME levels at BP of 80% Aviagen 2007 recommendation, but did increase at BP 100% and 120% Aviagen 2007 recommendations. The ME levels which elicited an increase in BW were slightly lower in the Starter than those used in the present trial, thus not having reached maximum requirement even at the highest ME level. Classen (2013) tested even lower BP levels (70%, 80% and 90% BP Aviagen recommendation) at 11.30 MJ/kg to 12.97 MJ/kg ME. While BW increased at 90% BP, no response to ME was observed at 80% BP, indicating that all ME levels were adequate for the low BP levels available. In agreement with these findings, Cho (2011) observed that increasing dietary energy levels resulted in reduced BW at low Lys levels. Moderate Lys levels did not show any difference with changing ME levels, but at high Lys levels, BW increased with increasing ME levels.

Like BW, BWG on day three was promoted by the lowest TLys and highest ME level. TLys1.4 was optimal from days 7 to 14 but reverted to TLys1.3 on day twenty-one. An inversed effect was seen in the carryover period with optimal BWG resulting from TLys1.6 and TLys1.7 in combination with ME 11.30 on days 28 and 35, respectively. This increased BWG was not sufficient to compensate for the lower BWG during the treatment phase. For the greater extent of the trial, no significant differences were observed between ME levels at TLys1.7. BWG at ME 12.97 did not respond to an increase in Lys beyond TLys1.4, with BW at ME 11.30 and ME 12.13 increasing at TLys1.7 from days seven to 14.

A similar trend was observed by Faulkner (1993), where BWG increased with increasing Lys levels until a point of plateau, beyond which BWG decreased at the highest Lys intake. This was attributed to a lack of energy which was required for protein deposition of that magnitude and possibly the higher energy cost of excess protein excretion. The possibility of Lys toxicity was also considered as an explanation for poor growth rates at the highest Lys level. In the present trial, BWG appeared to plateau at the higher TLys levels but no direct negative impact of excess Lys was observed. A further finding of Faulkner (1993) was that increasing energy to protein ratio increased the BWG up to a point, after which BWG declined. This was in contrast with current ME levels with the exception of BWG at TLys1.7, where increasing ME levels actually decreased BWG. The surplus of energy in the diet was presumed to have been directed towards higher fat deposition relative to protein deposition, and may have increased the metabolic heat increment which may have reduced FI and thus BWG.

Lemme *et al.* (2003) used Lys:AME as an indication of Lys levels across treatments. No difference was observed in BWG at the lowest Lys:AME between energy levels, which was in contrast to the present study, where even at TLys1.3, BWG increased with ME from the lowest to the highest ME level. Lemme *et al.* (2003) also observed that at 12.55 MJ/kg ME, the increase in BWG in response to increasing Lys:AME was more pronounced than at 13.18 MJ/kg ME, where BWG increased only up to a point before decreasing. This inverse to the findings of the present study, where BWG at the lowest ME level was more responsive to higher TLys levels.

5.2 Feed intake

Feed intake on day three was optimised at TLys1.3 and ME 12.13, this changed to TLys1.4 and ME 11.30 on days seven and 10. From days 21 to 28, FI was highest at TLys1.7 and ME 11.30 before reverting to TLys1.4 at day 35 with no significance between ME levels, thus despite a response in FI to increasing TLys levels, carryover effects were not significant at the highest TLys level, nor at different ME levels. This may be a similar response pattern as was observed by Chang *et al.* (2015), where at constant ME levels, FI increased with increasing BP levels from 80% to 100%, but decreased from 100% to 120% Aviagen 2007 recommendations. In a trial by Madsen *et al.* (2010), both ME levels of 95% and 100% Aviagen 2002 recommendations with increased BP levels from 95% to 100% Aviagen 2002 recommendations caused FI to decrease in broilers from 42 to 56 days of age. Reduced protein intake was however compensated for by high dietary protein content. This result is consistent with the findings at the lower TLys levels in the present trial.

For the greater extent of the trial, no significant differences were observed between ME levels at TLys1.4 and TLys1.7 or between TLys levels at ME 12.97. It was postulated by Classen (2013) that feeds with moderate to high levels of AA do not elicit a change in FI according to ME levels, as increased energy

may allow for AA to be spared from catabolism and direct them towards protein synthesis. At the lower TLys levels, FI was often observed to increase with increasing ME levels. This response was consistent only at TLys1.3 and TLys1.6 from days 21 and beyond. Faulkner (1993) observed that FI was lowest when birds were fed high Lys concentration feed, and FI increased with decreasing Lys levels. When birds were supplemented with sucrose, FI intake increased to a point then decreased with increasing Lys levels, which corroborates the current finding. Decreased FI in response to increasing energy to protein ratios may be resultant of an energy surplus thus an imbalanced diet leading to a higher metabolic heat increment. The expected reduction in FI in response to increasing ME levels was observed only at TLys1.7. This effect was consistent with those of Madsen *et al.* (2010), where increasing ME from 95% to 100% Aviagen 2002 recommendations decreased FI at both 80% and 115% BP Aviagen 2002 recommendations and Chang *et al.* (2015), where FI decreased with increasing ME levels from 80% to 120% ME Aviagen 2007 recommendations at constant BP levels.

5.3 Feed conversion ratio

The feed conversion ratio on day three was optimised at TLys1.7 and ME 11.30, this changed to TLys1.6 and ME 12.97 from days seven to 14. Optimal FCR stabilised at TLys1.4 on day 21 with no difference between ME levels. Carryover effects persisted at days 28 and 35, where FCR was optimal at TLys1.4 and ME 12.13. For the greater extent of the trial, no significant differences were observed between ME levels at TLys1.4. FCR improved with increasing ME levels as a general trend throughout this study. Response to TLys levels were rarely observed beyond TLys1.4. These findings agree with those of Madsen *et al.* (2010), where FCR was impaired with reduced ME levels but was improved with increased BP, and Chang *et al.* (2015) who observed an improvement in FCR when ME increased from 85% to 92% ME at 100% and 120% BP Aviagen 2007 recommendation.

5.4 Performance efficiency factor

The performance efficiency factor was optimised at TLys1.4 throughout the greater extent of the trial, with the higher ME levels being favourable. At levels exceeding TLys1.4, almost no improvement was observed, with TLys1.6 often yielding the least favourable results at ME 11.30. These trends from the treatment phase persisted throughout the carryover phase until slaughter.

5.5 Carcass composition

The highest carcass fat and lowest protein was observed at TLys1.3 and ME 12.13, while the highest carcass protein was observed at TLys1.7 and ME 11.30. Carcass fat decreased with increasing TLys levels

across all ME levels, with the lowest fat content at TLys1.3 and ME 11.30. No consistent trend was noticed with increasing ME levels. Carcass protein decreased with increasing ME levels at TLys1.7, and increased with increasing ME levels at lower TLys levels. An increasing trend in carcass protein was observed with increasing TLys levels, with the most distinct effect at ME 11.30. In this study, carcass fat and protein was analysed on broilers at 21 days of age so as to determine the direct treatment effect on these parameters. Current findings were in agreement with that of Chang *et al.* (2015), where abdominal fat increased with increasing ME levels and decreasing BP levels, with this effect being most pronounced at 80% BP Aviagen 2007 recommendation. Findings by Lemme *et al.* (2003) indicated that breast meat yield increased with increasing AA supply, with marked differences at the lowest Lys to AME ratio.

At higher ME levels, breast meat yield decreased at the highest Lys level. Abdominal fat pad was decreased by decreasing AME:Lys, with results most pronounced at the lower ME levels. This may be supported by the theory of Faulkner (1993), in which he stated that birds on higher energy to protein ratio diets over-consumed energy with less efficient usage of energy resulting from increased fat deposition. Additional findings by Chang *et al.* (2015) that are similar to findings of the present trial include carcass and breast meat yield being highest at 85% ME and 100% and 120% BP Aviagen 2007 recommendation, respectively. Both parameters decreased with increasing ME levels at 85% BP Aviagen 2007 recommendation. In contrast to present findings, lowest abdominal fat was observed at 85% ME and 120% BP Aviagen 2007 recommendation.

Classen (2013) fed diets ranging from 11.30 MJ/kg to 12.97 MJ/kg ME at 70%, 80% and 90% BP Aviagen 2007 recommendation. At 90% BP Aviagen recommendation, BW, carcass and breast meat yield and FCR were all favoured by increasing ME levels. No response was observed to ME at 80% BP Aviagen recommendation, indicating that all ME levels were adequate for the low BP levels available. At 70% BP Aviagen recommendation, FI, BWG and carcass and breast meat yield decreased in response to increasing ME levels, possibly due to large imbalance between energy to protein ratio.

Dozier *et al.* (2007) fed birds from 42 to 56 days of age diets with different ME and AA levels. Lower ME levels had better breast meat yield than those fed moderate levels of ME, possibly attributed to the increased Lys intake. High ME levels were observed to increase BWG, decrease FI and thus improve FCR. Feeding higher levels of AA, decreased FI and FCR, and increased breast meat yield. This experiment demonstrated the importance of AA density even at late stages in the growth as a tool to increase breast meat yield and improve return on investment.

Chapter 6
Conclusion

The objective of the current trial was to examine the effects of increasing TLys levels across various ME levels while simultaneously examining the effects of increasing ME levels across various TLys levels. The ME levels used were 11.30, 12.13 and 12.97 MJ/kg. The TLys levels used were 1.3%, 1.4%, 1.6% and 1.7%. These treatments were restricted to the Pre-starter and Starter feeding phases (days 0 to 21 of age) so as to determine the requirements of the early growing phase that would yield positive carryover effects on slaughter weight. The focus of the study was therefore on results at day three5, the termination of the trial.

No improvement was seen in BW by increasing TLys levels above TLys1.4 at any ME level. BW was optimised at TLys1.4 with no differences between ME levels. At the lowest TLys level, however, BW improved with every increase in ME level. This suggests that from TLys1.4, even the lowest ME level was adequate for maximum BW to be achieved. Similarly, it may be assumed that TLys1.4 was adequate for maximum BW across all ME levels. BWG was similarly favoured by TLys1.4 during the experimental feeding phase but an inversed effect was observed in the carryover phase, where improved BWG was noted at high TLys levels and low ME levels. This increased BWG was not sufficient to compensate for the lower BWG for these treatments during the treatment phase.

Feed intake did not strictly adhere to the expected trend of decreasing with each increase in ME level. While this did occur occasionally, FI was more influenced by increasing TLys levels, with increased FI as TLys increased from TLys1.3 to 1.4 at ME 11.30 and 12.13. Resultant 35 day FCR did not improve beyond TLys1.4 and differences due to ME levels were only observed at the lowest and highest TLys levels (ME 11.30 being the least favourable). No treatment effects were observed on the mortality rate.

The performance efficiency factor of broilers (calculated using BW, cumulative mortality rate and cumulative FI) was maximised at TLys1.4 with no differences between ME levels; however, these results were matched when TLys1.3 was combined with ME 11.30. Carcass fat was observed to decrease with increasing TLys levels across all ME levels, with the single results conforming to expected norms being an increase in fat content with ME increasing from 11.30 to 12.13. Contradictory trends were seen at other ME and TLys combinations. Carcass protein conformed to expected patterns by increasing with increasing TLys levels, however, increasing ME levels also resulted in increased carcass protein (with the exception of this trend at TLys1.7).

Scientists have observed carryover effects in BWG and FCE across the entire growing period when BP levels were increased in the Starter phase. This only held true when TLys increased from 1.3% to 1.4% in

the current trial. Recent findings have additionally indicated that at high Lys levels, increasing the energy content of the diet yielded increased values for BW, FE, and carcass and breast meat yield, despite there not being any significant difference in FI. This implies that at higher levels of dietary Lys, more energy would be required for optimal broiler performance.

In the current trial, results conformed to these findings as BW increased with an increase in ME at TLys1.3 and FCR improved with an increase in ME from 11.3 to 12.13 at both TLys1.3 and 1.7. While FI was not influenced by dietary energy levels, increased energy levels did not elicit a positive response in broiler performance except at the lowest TLys level examined. No consistent response was observed in reduction of FI at increased ME levels but rather FI increased with increasing TLys levels from 1.3 to 1.4 at ME 11.3 to 12.13 This is in agreement with recent findings that contradict the widely accepted notion of reduced FI resulting from high energy density diets, and thus increasing the AA density requirement.

Another popular theory that has recently been contradicted is that low ME levels in combination with high BP levels will result in reduced abdominal fat accretion and increase breast meat yield. However, carcass composition results of the current trial were correlated with past findings. A reduction in carcass fat and Carcass fat increased with increased ME from 11.3 to 12.31 and decreased with increased TLys levels. An increase in carcass protein was also observed at higher TLys levels.

The current marketing value of broilers is based on whole birds, thus relatively lower priority was placed on specific carcass components for this trial. Contradictory results require further research to be conducted in this regard. Results based on broiler performance suggest that TLys of 1.4% in combination with ME levels as low as 11.30 MJ/kg may be successfully used in the early feeding phase.

Chapter 7
Critical Review and Recommendations

This trial had multiple shortcomings as listed below:

1. Due to the large number of treatments in this trial, replicates of treatments were set at a maximum of four. A better indication of treatment effects may have resulted from a larger trial capacity, as this may have reduced the variability of results and thereby increased the significance as well as confidence levels.

2. Chicks were placed as-hatched according to commercial broiler rearing practices, this may have concealed different responses of male and female birds.

3. Trial feeds were produced at a commercial feed plant with production of 4 tonne batches per treatment. Feeds could have been more closely matched to treatment formulations if raw materials were weighed by hand and mixed in batch sizes similar to the required volume of feed for each treatment.

4. Due to technical difficulties, feeds were not delivered timeously to the trial facility, and thus may not have warmed sufficiently for maximal feed intake on day zero.

5. While dietary energy levels were formulated on ME basis, direct analysis of ME on the feeds was not conducted. Proximal analysis included crude fat, fibre, protein, ash, mineral analysis, moisture and starch content. The ME value of these feeds may only be roughly approximated as not every feedstuff used had an assigned ME contribution nor was the possible interaction effect between feedstuffs taken into account.

6. Freeze drying of 21 carcasses for carcass analysis was done in batches of 20 samples and this may have exposed different treatments to variable environments.

7. It recommended that more research should be conducted on nutrition in the early feeding phase, with only carryover effects examined in the remaining feed phases. This will allow for nutritionists to motivate that the increased cost of Pre-starter and Starter diets is a long term investment.

Chapter 8

References

AOAC, 1999. Official methods of analysis of AOAC. International 17th edition. Gaithersburg, USA.

AOAC, 2000. Official methods of analysis of AOAC. International 17th edition. Gaithersburg, USA.

Aviagen, 2007. Ross 308 Broiler: nutrition specifications. In-house publication, Global.

Aviagen, 2014. Ross 308 Broiler: nutrition specifications. In-house publication, Global.

Aviagen. 2015. Ross broiler pocket guide. http://eu.aviagen.com.

Baéza, E. & Le Bihan-Duval, E., 2013. Chicken lines divergent for low or high abdominal fat deposition: a relevant model to study the regulation of energy metabolism. Animal 7 (6), 965-973.

Baker, D. H., 1996. Advances in amino acid nutrition and metabolism of swine and poultry. Pages 41-53 in Nutrient management of food animals to enhance and protect the environment. E. T. Kornegay, ed. Lewis Publishers, New York.

Baker, D. H., 2009. Advances in protein-amino acid nutrition in poultry. Amino Acids 37(1), 29-41.

Batal, A. B. & Parsons. C. M., 2004. Utilization of various carbohydrate sources as affected by age in the chick. Poult. Sci. 83, 1140-1147.

Bellaver, C., Guideni, A. L., de Brum, P. A. R. D. & Rosa, P. S., 2002. Estimates of requirements of metabolizable Lys and energy in broilers from 1 to 21 days of age, using multivariate canonical variable. Bras. Zootec. 31, 71-78.

Brito, A. B., Stringhini, J. H., Gomez Xavier S. A., Cafe M. B., Andrade, M. A. & Leandro, N. S. M. R., 2011. Performance of broilers fed digestible amino acids based diets obtained from cecectomized roosters and broilers. Bras. Zootec. 40(11), 2400-2404.

Bryden, W. L., Hew, L. I. & Ravindran, V., 2000. Digestible amino acid values: variation and application. Aust. Poult. Sci. Symp. 12, 51-58.

Chang, A., Elfick, D., Sooncharernying, S. 7 Cerrate, S. 2015. Performance of Ross 308 broilers fed different levels of energy and balanced protein under moderate heat stress. Aust. Poult, Sci. Symp. 26, 117-120.

Cho, M., 2011. The impact of diet energy and amino acid content on the feed intake and performance of broiler chickens. MSc Thesis. University of Saskatchewan, Saskatoon.

Classen, H. L., 2013. Response of broiler chickens to dietary energy and its relationship to amino acid nutrition. Aust. Poult. Sci. Symp. 24, 107-114.

Collin, A., Malheiros, R. D., Moraes, V. M. B., Van As, P., Darras, V. M., Taouis, M., Decuypere, E. & Buyse, J., 2003. Effects of dietary macronutrient content on energy metabolism and uncoupling protein mRNA expression in broiler chickens. Br. J. Nutr. 90, 261-269.

Corzo, A., Moran, E. T. J. & Hoehler, D., 2003. Arginine need of heavy broiler males: Applying the ideal protein concept. Poult. Sci. 82, 402-407.

Corzo, A., Kidd, M. T., Dozier, W.A., Shack, L.A. & Burgess, S.C., 2006. Protein expression of pectoralis muscle major in chickens in response to dietary methionine status. Br. J. Nutr. 95, 703-708.

Dozier,W. A., Corzo, A., Kidd, M.T. & Branton, S.L., 2007. Dietary apparent metabolizable energy and amino acid density on effects on growth and carcass traits of heavy broiler. J. Appl. Poult. Res. 16, 192-205.

Eits, R. M., Kwakkel, R. P., Verstegen, M. W. A., Stoutjesdijk, P. & Greef, K. H., 2002. Protein and lipid deposition rates in male broilers chickens: separate responses to amino acid and protein-free energy. Poult. Sci. 81, 472-480.

Eits, R. M., Kwakkel, R. P., Verstegen, M. W. A. & Emmans, G. C., 2003. Responses of broiler chickens to dietary protein: effects of early life protein nutrition on later responses. Br. Poult. Sci. 44, 398-409.

Emmans, G. C.,1987. Computers in animal production. Br. Society of Animal Prod. 5,103-110.

Emmerson, D. A., 1997. Commercial approaches to genetic selection for growth and feed conversion in domestic poultry. Poult. Sci. 76, 1121-1125.

Emmert, J. L. & Baker, D. H., 1997. Use of the ideal protein concept for precision formulation of amino acid levels in broiler diets. J. Appl. Poult. Res. 6, 462-470.

Fan, H. P., Xie, M., Wang, W. W., Hou, S. S. & Huang, W., 2008. Effects of dietary energy on growth performance and carcass quality of white growing pekin ducks from two to six weeks of age. Poult. Sci. 87, 1162-1164.

Faulkner, A. S., 1993. The influence of dietary energy on the efficiency of utilisation of dietary protein in broiler chickens. Masters Agricultural Thesis. University of Natal, Pietermaritzburg.

Fernandez, S. R., Zhang, Y. & Parsons, C. M., 1995. Dietary formulation with cottonseed meal on a total amino acid versus digestible amino acid basis. Poult. Sci. 74, 1168-1179.

Garcia, A. R., 2006. Evaluating feed components for formulation of Pre-starter diets for broiler chickens. PhD Dissertation. University of Georgia, Athens.

Gous, R. M., Emmans, G. C. & Fisher, C., 1992. The response of broilers to feeds differing in protein content following a period of fattening. Thirteenth annual meeting of the Southern Poult. Science Society, Atlanta, Georgia.

Greenwood, M. W, Cramer, K. R., Beyer, R.S., Clark, P. M. & Behnke, K.C., 2005. Influence of feed form on estimated digestible lysine needs of male broiler from sixteen to thirty days of age. J. Appl. Poult. Res. 41, 130-135.

Grisoni, M. L., Uzu, G., Larbier, M. & Geraert, P. A., 1991. Effect of dietary Lys level on lipogenesis in broilers. Reprod. Nutr. Dev. 31, 683-690.

Han, Y. & Baker, D.H., 1993. Effects of excess methionine or Lys for broilers fed a corn-soyabean meal diet. Poult. Sci. 72, 1070-1074.

Havenstein, G. B., Ferket, P. R., Scheideler, S. E. & Rives, D. E., 1994. Carcass composition and yield of 1991 vs 1957 broilers when fed “typical” 1957 and 1991 broiler diets. Poult. Sci. 73(12), 1795-1804.

Havenstein, G. B., Ferket, P. R. & Qureshi, M. A., 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82, 1509-1518.

Hetland, H., Choct, M. & Svihus, B., 2004. Role of insoluble non-starch polysaccharides in poultry nutrition. Worlds Poult. Sci. J. 60(4), 415-422.

Holsheimer,J. P. & Ruesink, E. W., 1998., Effect on performance, carcass composition, yield and financial return of dietary energy and Lys levels in Starter and Finisher diets fed to broilers. Poult. Sci. 77, 806-815.

Iji, P. A., 1999. The impact of cereal non-starch polysaccharides on intestinal development and function in broiler chickens. Worlds Poult. Sci. J. 55(4), 375-387.

Kassim, H. & Suwanpradit, S., 1996a. The effect of dietary protein levels on the carcass composition of Starter and Grower broilers. Asian Australas. J. Anim. Sci. 9, 261-266.

Kassim, H. & Suwanpradit, S., 1996b. The effect of dietary energy levels on the carcass composition of the broilers. Asian Australas. J. Anim. Sci. 9, 331-335.

Kidd, M. T., Kerr, B. J., Halpin, K.L., McWard, G.W. & Quarles, C. L., 1998. Lys levels in Starter and Grower-Finisher diets affect broiler performance and carcass traits. J. Appl. Poult. Res. 7, 351-358.

Kleyn, R., 2013. Dietary energy and protein. Pages 23-41 and 45-55. Chicken nutrition, a guide for nutritionists and poultry professionals. Context. Leicestershire, England.

Leeson, S. & Summers, J. D., 2001. Protein and amino acids. Pages 102 -175 in Scott's nutrition of the chicken. 4th rev. ed. University books. Ontario, Canada.

Lemme, A., 2007. How to adjust dietary amino acids to energy in broiler diets? Pages 1-17 in Amino News volume 8(2). Degussa AG, Hanau, Germany.

Lemme, A., Wijtten, P. J. A., Langhout, D. J. & Petri, A., 2003. Interaction between ideal protein and dietary energy in growing broiler chicken. Eur. Sypm. Poult. Nutr. 14, 304-305.

Lemme, A., Ravindran, V. & Bryden, W. L., 2004. Ileal digestibility in amino acids in feed ingredients for broilers. Poult. Sci. 60, 423-438.

Lemme, A., Kemp, C., Fisher, C., Kenny, M. & Petri, A., 2005. Responses of growing broilers to varying dietary energy and balanced amino acid levels. Eur. Symp. Poult. Nutr. 15, 465-468.

Lemme, A., Wijtten, P. J. A., van Wichen, J., Petri, A. & Langhout, D. J., 2006. Responses of male growing broilers to increasing levels of balanced protein offered as coarse mash or pellets of varying quality. Poult. Sci. 85, 721-730.

Liu, S. Y., Raubenheimer, D., Selle, P. H., Gous, R. M., Hargreave, G., Simpson, R. J., Cadogan, D. J. and Cowieson, A.J., 2016. Protein and energy ratios influence performance in broiler chickens. Aust. Poult. Sci. Symp. 27, 170-172.

Lilburn, M. S., 1998. Practical aspects of early nutrition for poultry. J. Appl. Poultry Res. 7, 420-424.

Macleod, M. G., 1990. Energy and nitrogen intake, expenditure and retention at 20° in growing fowl given diets with a wide range of energy protein contents. Br. J. Nutr. 64, 625-637.

Macleod, M. G., 1991. Fat deposition and heat production as responses to surplus dietary energy in fowls given a wide range of metabolisable energy: protein ratio. Br. Poult. Sci. 32, 1097-1108.

Macleod, M. G., 1992. Energy and nitrogen intake, expenditure and retention at 32°C in growing fowl given diets with a wide range of energy protein contents. Br. J. Nutr. 67(2), 195-206.

Macleod, M. G., 1997. Effects of amino acid balance and energy: protein ratio on energy and nitrogen metabolism in male broiler chickens. Br. Poult. Sci. 38, 405-411.

Madsen, T. G., Carroll, S., Kemp, C. & Lemme, A., 2010. Influence of energy and balanced protein levels in wheat-based diets on Ross 308 broiler performance. Aust. Poult. Sci. Symp. 21, 95-98.

Malheiros, R. D., Moraes., V. M. B, Collin, A., Janssens, G. P. J., Decuypere, E. & Buyse, J., 2003. Nutrition Research. 23, 567-578.

Mariotti, F., Tome, D. & Mirand, P. P., 2008. Converting nitrogen into protein beyond 6.25 and Jones' factors. Crit. Rev. Food Sci. Nutr. 48, 177-184.

Nasr, J. & Kheiri, F., 2011. Effect of different Lys levels on Arian broiler performances. Ital. J. Anim. Sci. 10, 170-174.

Nesheim, M. C., 1968. Kidney arginase activity and Lys tolerance in different strains of chickens selected for a high or low requirement of arginine. J. Nutr. 95, 79-87.

Nieto, R., Prieto, C., Fernández-Fígares, I. & Aguilera, J. F., 1995. Effect of dietary protein quality on energy metabolism in growing chickens. Br. J. Nutr. 74, 163-172.

Nir, I., 1995. The uncertainties of broiler growth. Europ. Symp. Poult. Nutr. 10, 19-20.

Noblet, J., van Milgen, J. & Dubois, S., 2010. Utilisation of metabolisable energy of feeds in pigs and poultry: interest of net energy systems. Aust. Pout. Sci. Symp. 21, 26-35.

Noy, Y. & Sklan, D., 1997. Posthatch development in poultry. J. Appl. Poult. Res. 6, 344-354.

NRC, 1994. Nutrient requirement of poultry. 9th rev. ed. Natl. Acad. Press. Washington. D. C.

Okumora, J. & Mori, S., 1979. Effects of deficiencies of single amino acids on nitrogen and energy utilisation of chicks. Br. Poult. Sci. 20, 421-429.

Parsons, C. M., Potter, L. M. & Bliss, B.A., 1982. True metabolisable energy corrected to nitrogen equilibrium. Poult. Sci. 61, 2241-2246.

Pesti, G. M., 2009. Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. J. Appl. Poult. Res. 18, 477-486.

Pesti, G. M. & Fletcher, D. L., 1984. The responses of male broiler chickens to diets with various protein contents during the Grower and Finisher phases. Br. Poult. Sci. 25, 415-423.

Pirgozliev, V., Bedford, M. R., Acamovic, T., Mares, P. & Allymehr, M., 2011. The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens. Br. Poult. Sci. 52, 245-254.

Plumstead, P. W., 2005. Response of young broilers to graded levels of dietary protein and amino acids. MSc Thesis. North Carolina State University. Raleigh.

Plumstead, P. W., Romero-Sanchez, H., Paton, N. D., Spears, J. W. & Brake, J., 2007. Effects of dietary metabolizable energy and protein on early growth responses of broilers to dietary Lys. Poult. Sci. 86, 2639-2648.

Rabie, M. H. & Svilyagi, M., 1998. Effects of L-carnitine supplementation of diets differing in energy levels on performance, abdominal fat content, and yield and composition of edible meat of broilers. Br. J. Nutr. 80, 391-400.

Rostagno, H.S., Albino, L. F. T., Donzele, J. L., 2000. Brazilian tables for poultry and swine. Composition of food and nutritional requirements. Federal University of Viçosa, Viçosa, Brazil.

Samuels, M.L., 1989. Statistics for the Life Sciences. Collier MacMillian Publishers, London, UK.

Schutte, J. B., 1996. CVB-documentatie-rapport No. 18: Aminozuurbehoeften van leghennen en vleeskuikens (Amino acd requirements of laying hens and broilers). Central Bureau for Livestock Feeding (CVB), Lelystad, The Netherlands.

Statistical Analytical Systems, 2016. SAS User's Guide: Statistical Version 9.2 SAS Institute Inc. Cary, NC, USA.

Sibbald, I. R., 1976. The true metabolisable energy values of several feeding stuffs measured with roosters, laying hens, turkeys and broiler hens. Poult. Sci. 55, 1459-1463.

Summers, J. D. & Leeson, S., 1985. Broiler carcass composition as affected by amino acid supplementation. Can. J. Anim. Sci. 65, 717-723.

Swatson, H. K., Gous, R. M. & Iji, P. A., 2000. Biological performance and gastrointestinal development of broiler chicks fed diets varying in energy: protein ration. S. Afr. J. Anim. Sci. 30, 136-137.

Tavernari, F., Buteri, C. B., Rostagno H. S. & Albino, L. F. T., 2009. Effects of dietary digestible lysine levels on protein and fat deposition in the carcass of broilers. Bras.. J. Poult. Sci. 11: 99-107.

Tesseraud, S., Peresson, R., Lopes, J. & Chagneau, A. M., 1996. Dietary Lys deficiency greatly affects muscle and liver protein turnover in growing chickens. Br. J. Nutr. 75. 853-865.

Ullah, M. S., Pasha, T. N., Ali, Z., Saima, Khattak, F. M. & Hayat, Z., 2012. Effects of different Pre-starter diets on broiler performance, gastro intestinal tract morphormetry and carcass yield. J. Anim. Plant Sci. 22(23), 570-575.

Viera, S. L., Stefanello, C. & Cemin, H. S., 2016. Lowering the dietary protein levels by use of synthetic amino acids and the use of a mono competent protease. Anim. Feed Sci. and Tech. 221. 262-266.

Wijtten, P. J. A., Lemme, A., Pack, M. & Langhout, D. J., 2001. The effect of enhanced dietary "ideal" amino acid levels in maize- and wheat –based diets on performance of male and female broilers. Europ. Symp. Poult. Nutr. 13, 41-42.

Wijtten, P. J. A., Lemme, A. & Langhout, D. J., 2004a. Effects of dietary ideal protein levels on male and female broiler performance during different phases of life: single phase effects, carryover effects and interactions between phases. Poult. Sci. 83, 2005-2015.

Wijtten, P. J. A., Prak, R., Lemme, A. & Langhout, D.J., 2004b. Effect of different dietary ideal protein concentrations on broiler performance. Br. Poult. Sci. 45(4), 504-511.

Xie, M., Hou, S. S. & Huang, W., 2006. Methionine requirements of male white Peking ducks from twenty-one to forty nine days of age. Poult. Sci. 85, 743-746.

Yalcin, S., Özkul, H., Özkan, S., Gous, R., Yasa, I. & Babacanoglu, E., 2010. Effect of dietary protein regime on meat quality traits and carcass nutrient content of broilers from two commercial genotypes. Br. Poult. Sci. 51, 621-628.

Zhao, J. P., Chen, J. L., Zhao, G. P., Zheng, M. Q., Jiang, R. R. & Wen. J., 2009. Live performance, carcass composition and blood metabolite responses to dietary nutrient density in two distinct broiler breeds of male chickens. Poult. Sci. 88, 2575-2584.

Zubair, A. K. & Lesson, S., 1996. Compensatory growth in the broiler chicken: a review. Worlds Poult. Sci. J. 52, 189-201.

APPENDIX I

Temperature profile for Daybreak Trial Facility

Temperature (°C, 50 % relative humidity)			
Day	Lower temperature	Target temperature	Upper temperature
-1	34	35.5	37
-2	34	35.5	37
0	34	35.5	37
1	34	35.5	37
2	34	35.5	37
3	33	34.5	36
4	33	34.5	36
5	33	34.5	36
6	32	33.5	35
7	32	33.5	35
8	32	33.5	35
9	28.2	29.7	31.2
10	28.2	29.7	31.2
11	28.2	29.7	31.2
12	25.7	27.2	28.7
13	25.7	27.2	28.7
14	25.7	27.2	28.7
15	24.7	26.2	27.7
16	24.7	26.2	27.7
17	24.7	26.2	27.7
18	23.5	25	26.5
19	23.5	25	26.5
20	23.5	25	26.5
21	22.5	24	25.5
22	22.5	24	25.5
23	22.5	24	25.5
24	21.5	23	24.5
25	21.5	23	24.5
26	21.5	23	24.5
27	21.5	23	24.5
28	21.5	23	24.5
29	21.5	23	24.5
30	21.5	23	24.5
31	21.5	23	24.5
32	21.5	23	24.5
33	21.5	23	24.5
34	21.5	23	24.5
35	21.5	23	24.5

APPENDIX II

Lighting profile for Daybreak Trial Facility

	Controller set point		Daylight hours	Darkness hours
Day	Lights on	Lights off		
1	00:00	23:00	23:00	01:00
2	00:00	23:00	23:00	01:00
3	00:00	23:00	23:00	01:00
4	00:00	21:00	21:00	03:00
5	00:00	21:00	21:00	03:00
6	00:00	21:00	21:00	03:00
7	00:00	21:00	21:00	03:00
8	00:00	21:00	21:00	03:00
9	05:00	22:00	17:00	07:00
10	05:00	22:00	17:00	07:00
11	05:00	22:00	17:00	07:00
12	05:00	20:00	15:00	09:00
13	05:00	20:00	15:00	09:00
14	05:00	20:00	15:00	09:00
15	05:00	20:00	15:00	09:00
16	05:00	19:00	14:00	10:00
17	05:00	19:00	14:00	10:00
18	05:00	19:00	14:00	10:00
19	05:00	19:00	14:00	10:00
20	05:00	19:00	14:00	10:00
21	05:00	19:00	14:00	10:00
22	05:00	19:00	14:00	10:00
23	05:00	19:00	14:00	10:00
24	05:00	19:00	14:00	10:00
25	05:00	19:00	14:00	10:00
26	05:00	19:00	14:00	10:00
27	05:00	19:00	14:00	10:00
28	05:00	19:00	14:00	10:00
29	05:00	19:00	14:00	10:00
30	05:00	19:00	14:00	10:00
31	05:00	19:00	14:00	10:00
32	05:00	19:00	14:00	10:00
33	05:00	19:00	14:00	10:00
34	02:00	22:00	20:00	04:00
35	02:00	22:00	20:00	04:00