Feed intake and performance of Hubbard Flex broilers with varying dietary energy and protein concentrations

By

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I, Louma Mostert declare that the dissertation, which I hereby submit for the degree M.Sc. Agric at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature:……

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Dedication

I would like to dedicate this to my late Grandmother. Who was always overly enthusiastic about my career and studies. To my Grandfather, Chris van der Burgh, who's conversations I will treasure for ever.

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Abstract

Broiler breeding companies continue to analyse the recommended nutrient levels of broiler diets due to the short production cycle and continuous genetic development of broilers. Modern broilers reach marketable weights very early, often at an immature body weight and without achieving maximum genetic potential in terms of absolute quantities, for example daily growth. As broilers age, their daily feed intake increases and nutrients are deposited in body tissue, mainly as protein and fat. An imbalance of energy or protein cause for excess fat deposited in carcasses due to excessive feed intake to satisfy a nutritional requirement. The cost of feed contributes substantially to the total production expenses, with energy alone contributing approximately 70% of the total cost of poultry diets. Therefore, the focus of feed companies remain to determine the energy and protein requirements of broilers, in order to feed a balanced diet, delivering a bird with an optimal carcass composition.

Two experiments of identical design were conducted in floor pens to evaluate the effect of metabolisable energy levels as well as crude protein levels on broiler performance. The first trial was an energy dose-response trial and the second trial a protein dose-response trial. Each study was conducted over a period of five weeks where five thousand seven hundred and sixty Hubbard Flex (mixed sex as hatched) broilers were housed in 60 pens. Ninety six (96) chicks were randomly allocated in a pen at a stocking density of 16 birds/m^2. Each treatment was repeated once within a block, totalling to 10 replications/treatment. Water and feed were provided *ad libitum*.

In the energy dose-response experiment two iso-protein basal feeds were formulated and manufactured, one containing a high energy (HE) level, the other a low energy (LE) level. These basal diets were further diluted into four diets containing various percentages of energy (80% LE: 20% HE; 60% LE: 40% HE; 40% LE: 60% HE and 20% LE: 80% HE). The crude protein and amino acid balance was kept constant across all treatments. Six dietary treatment combinations were implemented in a 4-phase feeding programme: Pre-starter (0 to 10 d), Starter (11 to 18 d), Grower (19 to 28 d) and Finisher (29 to 35 d of age). Body weight (BW) and average daily gain did not show any significant difference between LE and HE treatments. A weekly numerical difference was recorded for BW between LE and HE treatments; where birds fed the HE diet weighed heavier on Day 35, at 1939.97 g compared to the 1898.10 g of birds fed the LE diet. FI increased as the energy concentration increased from the LE to HE treatments; and although the

differences in FI was non-significant (NS), birds fed HE diets consumed 2882.11 g/bird by Day 35 and birds fed LE diets, only consumed 2830.90 g/bird in total. No significant difference was recorded for cumulative feed conversion ratio (CFCR) among treatments by Day 35 and birds fed the LE diet ended with 1.53 points compared to 1.52 points for birds fed HE diet. Despite the dietary energy increase in energy level from the LE diet to the HE diet, there was no significant effect on daily mortalities; birds fed the LE diet showed 5.10% mortality compared to 5.41% mortality for birds fed the HE diet.

In the protein dose-response trial two isocaloric basal feeds were formulated and manufactured, one containing a high protein (HP) level, the other a low protein (LP) level. These basal diets were further diluted into 4 diets containing various percentages of protein (80% LP: 20% HP, 60% LP: 40% HP, 40% LP: 60% HP and 20% LP: 80% HP). The dietary metabolisable energy (ME) was kept constant across all treatments. Birds fed the HP diet showed the greatest BW at Day 21 with 900.97 g compared to birds fed the LP diet on 858.85 g; and weighed 1937.48 g at Day 35 compared to 1869.80 g for birds fed LP diet. Cumulative feed intake (CFI) decreased as the protein content in the feed increased. Although the results from Day 28 only approached significance, birds fed the HP diet consumed significantly less feed (2840.29 g/bird) than birds fed the LP diet (2913.66 g/bird) by Day 35. CFCR for HP was 1.50 points by Day 35 compared to 1.59 points for LP. The increase in protein content from LP to the HP diet, did not cause a significant increase in mortalities; although a numerical increase can be seen of 6.24% for birds fed on the LP diet compared to 9.06% mortality in birds fed the HP diet.

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List of Abbreviations

AA	Amino Acid
ACRBC	Athens-Canadian Random Bred Control
AME	Apparent Metabolisable Energy
ANOVA	Analysis of Variance
ARC	Agricultural Research Centre
BV	Biological Value
BW	Body Weight
BWe	Body Weight Empty
Ca	Calcium
CL	Control Line
CP	Crude Protein
CPR	Calorie: Protein Ratio
CVB	Centraal Veevoederbureau
Cys	Cystine
EAA	Essential Amino Acids
EFG	Emmans Fischer Gous
EL	Energy Level
ER	Energy Retention
ERd	Determined Gross Energy Retained
ERF	Energy Retained as Fat
ERP	Energy Retained as Protein
FCE	Feed Conversion Efficiency
FCR	Feed Conversion Ration
FI	Feed Intake
GIT	Gastrointestinal Tract
HE	High Energy
HP	High Protein
LE	Low Energy
Lys	Lysine
LP	Low Protein
ME	Metabolic Energy
Met	Methionine
MW	Metabolic Weight
N	Nitrogen
NEAA	Non-essential Amino Acids
NRC	National Research Council
NS	Non-significant
PL	Protein Level
Phos	Phosphorus
RE	Retained Energy
SE	Standard Error
SPL	Apparent Digestible AA poultry (Skynbare verteerbare amino sure pluimvee)
Thr	Threonine
TME	True Metabolic Energy
TSAA	Total Sulphur Amino Acids
QL	Quality-line

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CHAPTER 1

INTRODUCTION

The modern broiler was domesticated approximately 7000 to 10 000 years ago from the jungle fowl (*Gallus Gallus*). The early selection of heritable traits in poultry was mainly focused on egg laying. The industrialisation of agriculture in the early 20th century initiated the intensive genetic selection for the improvement of chicken meat, known today as broiler production characteristics (Schmidt *et al*., 2009).

The genetic improvement of the modern broiler is consequently one of the main factors that contributed to the significant development of broiler production in recent years (Marcota *et al*., 2008; Nir, 1998). At least 85% of the improvements noted in performance between 1959 and 2001 can be attributed to selection for body weight gain, feed conversion ratio (FCR) and breast meat yields (Vieira & Angel, 2012). Even today the poultry breeding industry continuously strive to improve the genetic selection for these favourable traits. These advancements warrant research that explores the improved strains, selected to maximise growth with excellent meat characteristics (Lopez *et al*., 2011). With the dramatic changes in broiler genetics, the selection for fast growth remained a major focus over the past 40 years; accompanied by the selection to consume feed at nearly full capacity regardless of the dietary energy level (Saleh *et al*., 2004).

Stimulated by the changing genetics and variation in environmental conditions, the recommended diet for the developing broiler is not absolute. Aside from the nutritional composition, the cost of feed contributes substantially to the total production expenses of a broiler operation; consequently, the optimisation of the dietary nutrient composition to achieve least cost production, has become essential for optimal profitability and minimising losses (Corzo *et al*., 2010).

In order to predict the feed intake of poultry could contribute significantly to achieve the optimum economic composition of feeds and feeding programmes. This could in turn create an opportunity to move away from the model of least cost formulation, using nutrient requirement tables (Gous, 2013). According to Emmans (1981), the consumption of feed by an animal is defined by either Voluntary Feed Intake (VFI) or appetite, which is the involuntary desire to consume a specific nutrient. Even so, unbalanced diets, toxic compounds and anti-nutritional factors create complex

interactions in the bird that have to deal with imbalances; it is therefore highly unlikely that feed intake can be successfully predicted using empirical equations (Gous, 2013).

Defining nutritional recommendations is essential for optimal growth and maximising carcass traits; therefore a considerable amount of research is dedicated to estimate the amino acid (AA) requirements for commercial broilers. However, often earlier growth stages enjoy more attention whilst research regarding late-finishing stages of broilers is somewhat scarce and past recommendations are often dependant on estimates from modelling rather than experimental data. Unfortunately, the inherent and unwanted variability in experimental data in the later stages of broiler studies lessen the possibility of accurate extrapolation of dietary recommendations (Corzo & Kidd, 2004). Data and knowledge obtained in later stages of dietary protein dose-response experiments as well as their effects, are often, but not always, ignored. The question therefore remains to what extent dietary history affects the response to dietary protein and energy levels (Eits *et al*., 2003).

Establishing the dietary metabolisable energy (ME) requirements is most important, since energy alone contributes approximately 70% of the total cost of poultry diets (Saleh *et al*., 2004); and energy has a major influence on the performance and body composition of a growing broiler (Lopez & Leeson, 2005). It would therefore remain favourable to determine the appropriate ME level which would result in optimal growth and feed efficiency, simultaneously providing a profitable production (Saleh *et al*., 2004).

In addition to dietary ME, determining the ideal protein concentration for broiler diets to maximise broiler performance and profit, requires basic knowledge of broiler performances and carcass quality, since both are related to dietary protein levels (Eits *et al*., 2003). This said, genotypic differences influence the response to varying dietary crude protein (CP) levels in young chicks; subsequently influencing the amino acids (AA) requirement as well (Sterling *et al*., 2006). Researchers have emphasised the importance of optimising dietary AA density in later feeding phases, when feed consumption is considerably greater, compared to younger birds (Corzo *et al*., 2010; Vieira & Angel, 2012).

Although energy and protein are two distinct nutrients with diverse functions, the relationship between the two nutrients needs consideration. As Malheiros *et al*. (2003) stated: “It is well known that dietary ME levels and CP content *per se* as well as their *ratio* have a major impact on broiler

performance in terms of growth rate, feed intake (FI), efficiency of conversion of feed into body weight gain and body composition." Numerous studies have established that as the dietary energy to protein ratio widens carcass lipids increase. The effect appears to be independent of energy sources since fat substitution for carbohydrates, at a constant energy to protein, has little effect on the carcass fat (Teeter *et al*., 1996).

Farrell (1974) reported numerously on the effects of dietary energy concentrations on the performance of growing chickens in the late 1950's, early 1960's, yet few recognised the importance of maintaining the energy to protein ratio in broiler diets. As a result, groups of chickens received deficient amounts of dietary protein and deposited excess carcass fat. This was a consequence of chicks that continued FI over a wide range of dietary energy levels to satisfy their protein requirement (Farrel *et al.*, 1973; Dalibard & Paillard, 1995).

In addition to the effect of dietary energy, higher energy levels may cause more rapid growth rates or even greater quantities of meat produced in a given time. Even so, raw material and production costs of higher energy diets in contrast to lower density diets may negate the benefits of improved performance of broilers noticed on the high energy diets (Saleh *et al*., 2004).

The aim of this dissertation was to continue focusing on the nutrient requirements of broilers subsequent to the rapid genetic change of broilers, thereby necessitating the continuous need to:

- Evaluate the effect of energy concentration on the feed intake and performance of Hubbard Flex broilers
- Evaluate the effect of protein concentration on the feed intake and performance of Hubbard Flex broilers
- Simultaneously attempting to determine the energy and protein requirement of Hubbard Flex broilers

CHAPTER 2

LITERATURE REVIEW

2.1 Modern Broilers

2.1.1 Genetics

a. The development of the modern broiler

Significant improvement in broiler strains were achieved by the selection for increased growth rate and feed efficiency; in 1950 broilers took 16 weeks to reach a marketable weight, whereas by 1990, this was reduced to 6 – 7 weeks (Schmidt *et al*., 2009). The outcome of the specialised selection have greatly increased the growth rate and reduced the feed conversion as well as the age to slaughter for commercial type broilers. Early genetic changes concomitant with management and nutritional alterations, as well as the application of efficient vertical integration led to the development of the modern broiler; which has the ability to produce poultry meat at about the same price today as in the early 1950s. These changes over the last 40 years have resulted in a modern broiler that requires approximately one-third the time (32 vs. 101d) and over a threefold decrease in the amount of feed consumed (1.47 vs. 4.42 FCR points) to produce a 1.8 kg broiler (Havenstein *et al*., 2003).

b. Genetic selection over time

Successful genetic selection programs in the breeder industry developed over time and are one of the reasons for the rapid growth and body conformation of modern broilers, especially in favourable traits such as greater breast meat yield (Scheuermann *et al*., 2003). Currently, genetic companies continue to carefully apply processes to improve broiler performance, especially since the selection for body weight (BW) changed the growth curve, increased feed efficiency and caused birds to reach market age earlier. However, various genotypes have different growth curves and therefore, different body compositions which result in altered nutritional requirements (Marcato *et al*., 2008).

The comparison between broiler strains are usually made at one or two processing ages, however, more complete information can be obtained by assessing and comparing the growth

curves for BW and breast weight. For example, the *Gompertz model* has been used for genotype comparisons among broilers, since the function explains the development of BW and different body components such as breast muscle (Scheuermann *et al*., 2003).

Most commercial broiler breeding programs are based on the selection of males, which are pre-selected for early BW. Selection responses with respect to growth, feed efficiency and feed intake are measured over time. However, in order to understand genetic relationship between performance traits such as growth rate, feed intake and feed utilisation efficiency, it is important to consider the direct and correlated responses to the selection of these traits (Pym, 2005).

Over 12 generation lines, chickens were selected for production traits such as (Pym, 2005):

- Increased 35-63 d weight gain
- Increased 35-63 d feed intake
- Decreased 35-63 d FCR

However, it was not until the mid-1980s that commercial broiler breeders incorporated direct measures of feed intake as selection criteria in their broiler breeding programs. Up until then, it was assumed that continuous selection for greater growth rates would result in an adequate response in feed efficiency, since the two traits are positively correlated. The direct measure at selecting for feed intake aired after reports in which researchers informed breeders that the considerable variation in feed efficiency, to a given BW, was not attributable to variation in growth rate alone (Pym, 2005).

Another possible method for the selection experiment aimed at improving broiler carcass quality, in terms of body composition, was the use of a so-called ‘quality-line’ (QL) selected for high breast meat development, low abdominal fat percentage and the highest possible BW. Concomitant to the QL, a control line (CL) should be kept in order to facilitate comparison between lines and to estimate actual genetic gain in each generation (Tesserand *et al*., 2003).

Some early geneticists developed random breeding populations using crosses of several early white-feathered broiler strains as the base population; for example, Athens-Canadian Randombred Control (ACRBC), which was established in 1957 and has been maintained at a Regional Breeding Laboratory. The ACRBC is still in existence and is an extremely valuable tool for measuring genetic change over time (Havenstein *et al*., 2003), similar to the QL and CL bases.

To confirm that the breeding program was successful, a regression at 56 days BW of the ACRBC generations was drawn and found that the regression did not differ significantly; indicating that the 56 day BW of the ACRBC was the same as in 1957 as it was at the time of the study conducted in 1991 (Havenstein *et al*., 2003).

However, differences in selection criteria among primary breeders, favouring specific environments or genotypes, may affect the chronological growth processes. Consequently, the differences between the growth curves of commercial broiler strains or even cross strains, could economically favour the use of a specific genotype or sex in order to reach the desired marketable BW or breast meat yield and weight (Scheuermann *et al*., 2003).

c. Assessment and selection criteria

Acceptable criteria for assessing the performance of differing broiler strains are (Lopez & Leeson, 2005):

- Growth rate (as a major focus in broiler industry)
- Body weight to age
- Feed conversion efficiency (FCE) or feed conversion ratio (FCR)
- Carcass composition (Smith & Pesti, 1998; Berhe & Gous, 2008)

Carcass composition (as protein accretion to fat deposit) is the key criterion for predicting different nutrient requirements such as energy intake and amino acid (AA) requirements among various strains (Mack *et al*., 1999).

However, the anticipated outcome of criterion trait selection would be noticed in the following measures (Mack *et al*., 1999):

- Increased BW
- Higher body weight-for-age or body weight gain (Leclercq & Guy, 1991; Lopez *et al*., 2007)
- Rapid/increased growth rates
- Desired body composition changes
- Increased FCE (Leclercq & Guy, 1991) or decreased FCR
- Robustness
- Disease resistance
- Absence of metabolic defects

Evidently, not all traits can be selected as a priority trait for every breed available and subsequently different breeds are known for sought after traits or a combination thereof. Different growth potential for various strains have also been found (Smith *et al*., 1998) and are true even under similar environmental and dietary conditions, for different breeds, strains and individuals (Pym & Farrel, 1977).

A variation of factors are a possible explanation for better feed efficiency in a broiler line, specifically selected for decreased FCR (Pym, 2005):

- A reduction in the energy content of the gain i.e. partitioning of water, fat and protein
- An increase in digestibility or metabolisability of dietary nutrients
- A reduction in daily maintenance requirements per unit liveweight
- An increase in the net availability of ME for gain
- An increase in the net efficiency of protein utilisation through a reduction in protein breakdown rate
- A reduction in external losses – i.e. feed spillage has to be considered

The body weight-to-age has also increased at the hand of maintained selection reaching market weight earlier but often at immature carcasses and, therefore, not achieving maximum genetic potential for protein or even fat deposition. Ultimately, immature broilers consume significant amounts of protein, but are often not capable of consuming enough energy to accomplish growth for normal body composition. On the contrary, at *ad libitum* feed levels, mature broilers with completed skeletal growth and muscle development continue consuming high volumes of energy; despite the high consumption volumes, the broilers also deposit the excess energy as fat (Boekholt *et al*., 1994). In the broiler industry, high quantities of carcass fat is generally consider as a waste (Boekholt *et al*., 1994) and an unfavourable trait, thus genetic selection and feeding programs aim at the limitation thereof (Lopez *et al*., 2007).

d. Consequence of continuous selection

Continuous selection for body weight gain has resulted in broilers that reach an earlier marketable weight and with specific carcass characteristics (Lopez *et al*., 2007). However, when comparing the modern broiler to an unselected heritage line in the 1950s, selection for rapid growth has affected many of the major organ systems, including (Schmidt *et al*., 2009):

- The digestive system
- The cardiovascular system
- Immune system
- Muscle system
- Nervous system

Leeson and Summers (1980) reported an increase in body weight of 1.85kg to 2.258kg in 42 day old broilers by 23% over 25 years from using a single diet throughout the experiment. Leaner birds were expected from the use of a single experimental treatment, since protein was reduced and energy increased in diets as birds aged (Lopez *et al*., 2007). Lopez *et al*. (2007) identified a continuation of fat and protein deposits, until the end of the experiment (day 42); even though the body energy content (as fat and protein) was relatively consistent throughout the 42 day growth period. Therefore, selection for reduced abdominal fat and greater feed efficiency produced leaner broilers; but, this would have been affected by varying dietary energy levels (Lopez *et al*., 2007).

Even though genetic selection has contributed substantially to the improved performance of the modern broiler, continuous selection for performance traits has been accompanied by unfavourable factors and traits. Broilers have been selected for increased growth rate over generations; but, the selection pressure on growth in commercial broiler breeding programs has been somewhat relaxed in more recent years to accommodate aspects of performance that have suffered because of the early high emphasis on juvenile growth rate (Pym, 2005).

Genetic change has also led to a broiler bird with an insatiable appetite, but at the same time a broiler that is highly efficient at converting feed to body mass along with increased muscle yield. Even so, undesirable traits such as these mentioned below, have developed, presumably due to the stress induced by such rapid growth (Schmidt *et al.,* 2009):

- Sudden death syndrome and ascites due to cardiovascular failure
- Reduced adaptive immune function or compromised immunocompetence
- Poor reproductive performance
- Leg and skeletal disorders
- Poor body conformation and composition
- High incidences of metabolic disorders

- Gluttony

Together with the above factors, undesirable traits developed over a period of time together with possible constraints (listed below) as a result of continued increase in growth rate (Gous, 2010):

- Maternal effect on egg size
- The rate at which the gastro-intestinal tract (GIT) matures
- Possible problem of supplying insufficient quantities of amino acids in the feed
- Growing inability of dissipating sufficient heat to the environment in order for the bird to grow at its genetic potential

e. Fast versus slow-gaining strains

The accretion of muscle protein, i.e. muscle growth, depends on a balance between synthesis and degradation of muscle proteins. Therefore, comparing a broiler-type (fast/heavy) and a layer-type (slow/light) chicken, would suggest that greater muscle development relies more on a decrease in protein degradation than on an increase in protein synthesis (Tesseraud *et al*., 2000).

When fast and slower-gaining animals grow to the same final BW, animals that grow at a slower rate contain less lipid and more protein when compared to fast growers. This is due to the primary building blocks of muscle protein being at a greater proportion of daily growth in slower-gaining animals, i.e. at increasing growth rates, the proportion of protein decreases (Corzo *et al*., 2004). However, it cannot be assumed that if energy intakes increase in magnitudes of accumulative levels that the energy will be retained less in protein deposition and more in fat tissue. Since each additional unit of energy retained has a constant composition between fat and protein retention; it can be concluded that the protein (as % of total Retained Energy (RE)) is much more affected at lower energy intakes (Boekholt *et al.*, 1994).

i GIT development

As the modern broiler developed, geneticists knowingly placed emphasis on the selection for rapid growth. Together with the increased genetic growth potential, nutritionists are formulating complete feeds to optimise or maximise growth of these lines, when fed *ad libitum*.

The development of the GIT immediately after hatch has a major role on the initiation of broiler growth. During this period the GIT grows more rapidly than the entire body and even more so in

a broiler than in slow strains, such as the Leghorn chicken. Nir *et al*. (1993) stated that the allometric growth of the intestinal content was much higher in broilers than in the Leghorn chicks, thereby confirming the assumption that juvenile broiler chicks eat at a rate nearing their own gut capacity.

ii Feed intake

Assuming that broiler chicks eat at a rate near to their own gut capacity, the FI of broilers at the age of 4 days reach a peak of 30% of BW. For a slow-strain Leghorn chick, FI only reaches a peak at the age of 6 days and 15% of BW (Nir *et al*., 1993).

When using the concept that the GIT is a limiting factor for FI, heavy-body chicks are more responsive than light-body chicks. For example, when an intubation technique was used to simulate overfeeding, light-weight chicks showed an increase in both lean and fat-tissue growth whereas broilers illustrated adaptive responses to overfeeding (Nir *et al*., 1993).

When overfeeding a broiler, an increase of approximately 30% in the duodenum weight was noticed in comparison with a slow-strain chicken. For the latter, an increase of 60% in duodenum weight was measured, showing a propensity towards a greater physical limit for feed intake compared to a fast-strain bird (Nir *et al*., 1993).

iii Digestion

Although broilers physically peak at a younger age than slow-gaining strains, the excessive feed intake does not compensate for a higher secretion of digestive enzymes as it would have been expected. The digestive enzyme activity was found to be higher than in the Leghorn although broilers hatch with a higher secretion of saccharases than the Leghorn. However, the enzymes declined much faster in a broiler and at the age of 7 days, the activity of the saccharase enzymes was noticeably greater in the Leghorn. Subsequently, the broiler chick would have to cope with a higher amount of chyme and in the same context, maltase might have been the limiting factor for carbohydrate absorption in broiler chicks. Apart from the relatively limited carbohydrate absorption, excessive intake in juvenile broiler chicks was followed by a lower digestibility of dietary fats and lower nitrogen retention within the first two weeks post-hatch (Nir *et al*., 1993).

iv Selective feeding

In the late 1970's researchers confirmed that domestic fowls could discriminate between diets varying in nutrients such as energy, protein and even amino acids. Nobel *et al.* (1993) stated that fast-growing broiler strains were more sensitive to differing essential AA levels than slow-growing egg producers.

When given a choice between high protein (HP) or low protein (LP) with high energy (HE), broilers preferred diets which allowed growth at a rate slightly lower than a diet assumed to be optimal for growth rate. Broilers may therefore, innately select a diet that has possible long-term survival benefits as well as maintaining a state of well-being, rather than selecting a diet with economic benefits at a marketable age (Nobel *et al.,* 1993).

v Growth rate

Possible measures to increase growth rate *per se* would include options such as starting with a larger day old chick. In order to produce heavier birds at slaughter, Gous (2010) suggested selecting for heavier day old chicks. Heavier day old chicks could either be achieved by increasing egg weight or increasing the growth rate of embryos during incubation. Even though the latter seems impossible, selection against small eggs laid by young broiler breeders are an option, since the embryonic development during the last stages may be restricted due to limited space within the eggshell.

Apart from the selection for heavier chicks, adult broilers have been continuously selected for increased growth rate by direct selection for increased live weight at a given age. A very prominent consequence of this type of selection is the substantial increased appetite (feed intake), but also a considerable improvement in feed efficiency; and a noticeable reduction in the age at processing (at the same body weights). The subsequent shorter growing period resulted in a commensurately reduced maintenance requirement (Pym, 2005).

2.1.2 Simulation models, mathematical equations, regressions and functions

According to Gous *et al*. (1999), fitting the bird's growth curve is the first step to predict the nutritional requirements of the different genotypes; thereby supporting the selection process and

contributing to the assessment of birds' genetic potential. Many non-linear mathematical models have been used to describe animal growth and body nutrient deposition or requirements.

Lopez *et al*. (2007) mentioned that most models are:

- Linear
- Quadratic regressions
- Allometrics
- The Gompertz equation

Models are used in modern research to quantify Energy Retention (ER), Energy Retained as Fat (ERF) and Energy Retained as Protein (ERP), and are either a function of time (quadratic equation) or a function of body weight (allometrics and The Gompertz eq.); both of which fit the data reasonably well within the first 42 days of production and could therefore, be used to predict ER, ERF and ERP from time or BW (Lopez *et al*., 2007).

a. The Gompertz equation

The Gompertz equation has conventionally been accepted in broiler studies to fittingly describe "growth over time, growth of the body components or both" (Lopez *et al*., 2007). The Gompertz equation describes a sigmoidal biological growth pattern of broilers with a slow *initial rate of growth*, followed by acceleration up to a certain age, known as the *point of inflection*, and followed by a subsequent *decline in the rate of growth* as body weight approaches its maximum value and reach maturity (Lopez *et al*., 2007).

Usually, the methods for evaluating the growth of body components in broiler studies using the Gompertz equation have been based on data obtained from birds between 0 and 10 to 16 weeks of age. In these situations, measurements are obtained beyond the inflection point and the asymptotic values for BW or body components are estimated with rational accuracy (Lopez *et al*., 2007).

However, modern broilers reach slaughter BW earlier (approximately 6 weeks) and likely without achieving maximum genetic potential for fat and protein deposition. Therefore, the asymptotic values for BW or body components are unlikely to be predicted accurately when measurements

are not obtained beyond 42 to 49 days of age. This is reflected in the high standard error (SE) values for estimates of these parameters (Lopez *et al*., 2007).

b. Quadratic regression equations

As a result of high SE values and the challenges with the early development of broilers, the use of equations to predict growth performance of modern boilers up to 42 days of age, may be as effective as the equation in representing growth patterns.

The Gompertz equation could be considered more of a biological model to describe growth patterns instead of the polynomial approach. The Gompertz equation could also be used to relate body constituents such as ERF and ERP to body weight. This is, relatively more an experiential application, since it implies that body weight gain can continue after ERF and ERP have reached their plateau values. Thus, the development of the equation in such an instance is only applicable to body weight ranges that are evaluated in an existing study and cannot be used reliably to deduce values for birds of heavier weights (Lopez *et al*., 2007).

2.1.3 Body composition

a. Body development phases

Broiler growth is characterised by a sigmoidal curve with three complement phases (Scheuermann *et al*., 2003):

1. An initial exponential development phase
2. An intermediate or transitory phase
3. And a final phase of inhibited growth consisting of a gradual reduction in the growth rate following an asymptotic increase in the body weight

b. Body weight gain

Live body weight gain is fundamentally the deposition of feed nutrients in body tissue, mainly as protein and fat; yet, with water selected with intent in order to reach market weight earlier and with specific desired carcass characteristics. It is expected that as BW increases the quantities of body fat and protein would also increase. This said, due to the nature and relationship between body

fat and protein, they increase at different rates, with fat depositing at potentially increasing rates at matured age and subsequently a declining protein accretion at an older age (Boekholt *et al*., 1994; Lopez *et al*., 2007)

The relationship between protein and fat in the body is influenced by (Lopez *et al*., 2007):

- Genetics / Genotype
- Nutrition
- Sex
- Body weight or degree of maturity
- Environmental conditions

In all likelihood, the efficiency of transfer of energy from feed to tissue differ between fat deposition and protein accretion and changes in the proportion of both fat and protein during growth influence the total energy in the body, as well as the efficiency of body weight gain. Modern broilers reach a desired commercial BW early and at an immature body weight, often without achieving maximum genetic potential for fat or protein deposition in terms of absolute quantities deposited each day (Lopez *et al*., 2007).

Energetic efficiency of ME used for tissue gain is dependent on various factors but such efficiency has been shown to vary with substrate source. Utilisation of protein or fat for tissue gain depends on the biological value of the protein sources as well as the fat used for lipogenesis (Teeter *et al*., 1996). The deposition and efficiency of energy utilisation will be discussed in more detail in Section 2.3.2 (c).

c. Protein utilisation for growth

It is commonly assumed that efficiency of EAA utilisation for growth does not differ between differing genotypes within one species. This includes the assumption that the digestion and absorption rate are not affected by the genotype. The question remains whether the long-term selection of broiler chicks for improved meat yield may be, in part, the consequence of a higher ability to utilised limited amino acids. Selection over many years caused changes in the proportion of muscle mass, the number of fibres per muscle and the diameter of muscle fibres, suggesting that protein turnover rates in the body differ among genetic strains of a species. Therefore,

changes in the ratio of synthesis and degradation of muscle protein may bear consequences for the efficiency with which amino acids are utilised (Fatufe *et al*., 2004).

From the experiment of Corzo *et al*. (2010), it was noted that carcass weight and carcass yield were unaffected by the dietary treatments, however, breast meat yield was sensitive to the amino acid density of the feed.

d. Carcass quality and fatness

The quantity of carcass fat is generally considered an unfavourable characteristic in broilers, leading to continuous research, genetic selection and feeding programs aimed at reducing or limiting carcass fat content (Lopez *et al*., 2007). The degree of carcass fatness is well known to be influenced by nutritional as well as non-nutritional factors such as (Lopez *et al*., 2007):

- Age
- Sex
- Ambient temperature

In general, carcass fatness will not change as long as the energy: protein (kJ ME: CP) ratio does not change. If the ratio is not constant, carcass fatness increases as the dietary energy level increases and the ratio widens (Teeter *et al*., 1996). Early discovery of this statement was reported by Bartov (1979), whereby increasing the dietary energy content through the addition of fat, without altering the energy: protein ratio, showed no effect on the carcass fat content of the broilers.

Except for paradoxical theories on the manipulation of carcass composition with genetics and dietary protein, there is also much debate regarding the influence that dietary energy levels or ME intake (Farrell *et al*., 1973) have on carcass composition and quality (low % of fatness). There are four general nutritional factors that will influence the amount or degree of body fat in broilers (Saleh *et al*., 2004):

1. Narrowing the energy: protein ratio generally prevented excessive deposition of body fat
2. An imbalance of AA may result in an increase in carcass fat
3. The specific effect of dietary fat on carcass composition
4. The effect of dietary energy levels on the direct degree of fatness of broiler carcasses

Therefore, with the average dietary energy level as a critical contributor to carcass characteristics, the effects of differing energy levels were clearly noticed in Saleh *et al.* (2004). Carcass weight improved significantly as the dietary energy level increased, but peaked at 3188 ME kcal/kg (13.3 ME MJ/kg), and declined at levels greater than 3344 and 3383 ME kcal/kg (14 ME MJ/kg and 14.1 ME MJ/kg). Dressing percentage was relatively equal to carcass weight and inclined up to 3188 ME kcal/kg (13.3 ME MJ/kg) after which the percentage began to decline.

Even though breast meat yield (as a percentage of carcass weight) showed significant differences among differing average energy levels, it did not follow any specific trend among treatments. Contrary, breast meat weight increased, with a peak weight at 3188 ME kcal/kg (13.3 ME MJ/kg), and declined as the average dietary energy exceeded 3304 ME kcal/kg (13.8 ME MJ/kg), very similar to the abovementioned characteristics (Saleh *et al*., 2004).

There was no consistent relationship between the average dietary energy level and abdominal fat weight or fat percentage (of the carcass), even though high dietary energy levels are often blamed for excessive abdominal fat accumulation. In this particular experiment treatments maintained a constant energy: protein ratio, thereby proving to have a greater effect on the carcass fat than dietary energy *per se* (Saleh *et al*., 2004).

Besides the influence of dietary energy, Dozier *et al*. (2006) stated that increased dietary amino acid density was shown to decrease abdominal fat percentages in broilers. In the particular study, the researchers discussed the effect of 3- and 4-phase schedules on the abdominal fat percentage. The researchers discovered that fat did not decrease significantly if the 3- or 4-phase diets had increased nutrient densities; still, with only moderate nutrient density diets in both phases, the 3-phase diet had less abdominal fat percentage than birds feeding on the 4-phase diet. The ratio of dietary energy to CP influenced the abdominal fat percentage directly. The greater energy: protein ratio of 176 kJ/ g in the 3-phase led to higher breast meat weights with a reported 5% increase in total white meat; compared to the 166 and 184 kJ/ g ratio from day 36 to 46 and day 47 to 56 in the 4-phase, respectively. The increase in energy: protein ratio fed during 47 to 56 days of the 4-phase diet could have caused the increase in fat.

Kidd *et al*. (2004) reported a 5% increase in total breast meat (pectoralis major and minor breast muscle) when broilers were fed high density diets compared to moderate nutrient densities. The response to growth rate and breast meat yield were more acute in the study of Kidd *et al*. (2004)

since the specification used for CP, lysine and total AA were even greater in the moderate scheduled diets than in the study reported by Dozier *et al*. (2006).

e. The consequence of overfeeding the modern broiler

Broilers and Leghorn-type chicks were force-fed and the differences between the breeds were noticed in the content of the GIT and the adipose tissue of the two strains. Due to a supposed increase in duodenum weight when a Leghorn-type bird was force-fed, efficient digestion of ingested feed resulted in both lean and fat-tissue growth. The amount of adipose tissue fat in an over fed Leghorn-type was similar to that of a broiler chick fed *ad libitum.* Broilers showed reluctance to overfeeding and this could possibly arise from the limited digestive ability of the adipose tissue (Nir *et al*., 1978).

Overfeeding caused for a surplus in fatty acids and glucose from excessive feed absorption as well as the unnatural accumulation of fat. However, when fat accumulated in excess of a certain limit, the adipocyte possibly resisted further incorporation of fatty acids and glucose; therefore, the reduced incorporation could have been contributed to a reduced absorption of feed from the GIT and eventually output of feed through the GIT (Nir *et al*., 1978).

In the same experiment, high residual crop content was found in broiler chicks before tube-feeding the next meal, preventing an excess of feed intubation. The residual GIT content could be the link between the disposition of broiler chicks being force-fed and the body fat content of the bird. Therefore, the adipose tissue may be involved in feed intake as well as the predisposition of broilers to digest excessive amounts of feed efficiently (Nir *et al*., 1978).

2.1.4 Males and females

It is a well-known nutritional fact that males and females have different dietary requirements, growth rates (Marcota *et al*., 2008), response to protein intake (PI), carcass yield (especially breast meat yield) and BW over the same time period (Smith *et al*., 1998). Males display greater BW (Scheuermann *et al*., 2003), growth rate, feed efficiency (Shalev & Pasternak, 1998), lysine requirement (Baker, 2008) and breast meat yield (Scheuermann *et al*., 2003).

However, under high ambient temperatures, body weight gain of males is depressed to a greater extent than females, leaving females with better feed efficiency and similar BW performance for that time. The energy required for body weight gain is the energy combusted in the accretion and deposition of body tissue, protein and fat respectively; thus an identical value is assigned to both male and females (Shalev & Pasternak, 1998).

The difference in nutrient requirement between males and females can therefore be attributed to the variation in maintenance requirements; where females generally have a lower basal metabolism rate in most commercial animals as well as in humans. The outcome of the study indicated that males required 13% more feed on average for maintenance per kg metabolic BW. The higher metabolic rate of males is probably regulated by primary male hormones (Shalev & Pasternak, 1998).

Leeson & Summers (1980) investigated the response to increasing energy: protein ratio of various body components in male and female broilers up to 70 days. As BW increased, fat and protein deposition increased, where the body fat content (as a percentage of the BW) increased more rapidly in males and females; compared to body protein, which remained fairly constant (Lopez *et al*., 2007).

2.1.5 Behavioural and social changes

Future enhancements in genetic improvement to broiler performance will continue; however, emphasis on welfare and the ability for broilers to strive in widely different environments, together with greater growth rates, are of immense importance (Nielsen, 2004).

Concomitant to strain variability, researchers also proposed that changes in (Nielsen, 2004):

- Anatomical
- Behavioural
- Physiological factors

were associated with continued selection for broiler performances. Examples of behavioural changes are decreased activity in fast growing strains and alteration to satiety mechanisms, which takes place in the brain. This leads to excessive and compulsive feed intake and behaviour,

eating to near-capacity under *ad libitum* conditions and without regard for the nutritional content of the feed (Nielsen, 2004).

There has to be a physical and physiological limit to the rate at which broiler birds can grow, even though, up to date no reduction in the progress rate has yet been indicated (Gous, 2010). Together with welfare and liveability of broilers, which should be thoroughly monitored as genetics improve and selection continues, producers are compelled to re-evaluate feeding regimes for broilers (Gous, 2010).

These regimes include factors, such as (Gous, 2010):

- Length of feeding phases
- Major nutrient inclusion values/amount
- Raw materials used and levels included
- Additives included
- Additional vitamins and minerals included

2.2 Feed intake

Fast growing modern broilers fed under *ad libitum* feeding conditions with continuous light, feed approximately 2-4 times per hour. The continuous intake is costly as soon as feed intake is in excess of the required amount for maintenance, growth and activities including thermoregulation. Excessive feed intake causes for a diet energy loss in the form of heat increment and increased fat deposition.

Emmans' (1981) theory stated that birds eat to meet their requirements subject to constraints. Therefore, assuming broilers do not eat more than they need, this would leave very little buffer, with regards to volume, to compensate for any nutritional deficits (Nielsen, 2004).

2.2.1 Feed intake regulation

Birds attempt to consume sufficient amounts of a provided feed to allow them to meet their nutrient requirements; firstly for maintenance followed by growth and production. Constraints such as bulky feed, the inability to lose sufficient heat to the environment or an imbalance in the nutrient content of feed may prevent sufficient feed intake. Birds have, however, evolved to

survive whilst developing the capability to select or regulate the intake from an array of foods. If a provided feed is unbalanced containing, for example, very low protein and high energy, birds will consume excessive amounts of energy in order to consume sufficient protein; and in the process deposit excess lipid (Gous, 2013).

a. Mechanisms

Once a feed is consumed, there are several possible mechanisms that regulate FI. The common regulatory mechanisms include (Ferket & Gernat, 2006):

- The Glucostatic Theory
- The Thermostatic Theory
- Distension of the Gastrointestinal tract (GIT)
- Circulating AA and PI
- The Lipostatic Mechanism

as well as (Ferket & Gernat, 2006):

- Genetics
- Dietary factors
- Environmental and management factors

The Glucostatic theory seems to have priority over other theories as birds tend to consume feed to satisfy their energy requirement first and secondly, to satisfy their daily AA requirements. Therefore, in commercial conditions feed intake is greatly influenced by both the dietary energy and amino acid profile (Ferket & Gernat, 2006).

The Lipostatic theory is considered irrelevant in the modern broiler breeds, since the birds do not become old enough for the theory to be proven. The theory explains the innate defence of the bird's body to have a certain body fatness set point and their intake of energy will increase to the point where this minimum body fat content is reached. However, modern broilers have been selected for body weight gain and high rate of feed conversion and broilers have apparently become hyperphagic in order to accommodate the genetic tendency for high body fat content (Ferket & Gernat, 2006).

b. Genetics

An anticipated consequence of continuous selection for improved growth rate over generations has been a substantial increase in appetite or VFI in modern broilers; combined with a considerable improvement in feed efficiency. The continuous selection for increased live weight at a given age was accompanied by a marked increase in feed intake (g/d), with a subsequent reduction in slaughter age at the same BW. The considerable improvement in feed efficiency and feed conversion was largely due to the shorter growing period and coinciding to reduced maintenance requirements (Pym, 2005).

c. Physiological factors

Physiological mechanisms are functional mechanisms that control the feed intake within the bird's internal processes, either by limiting the feed intake or encouraging the consumption of a particular nutrient with an energy yielding component. Alternatively, the limit could be physical, where the bird eats up to a maximum gut fill or gut capacity. Either way, these mechanisms require innate sensors which informs the bird of the ingested feed (Ferket & Gernat, 2006).

The hypothalamus is considered to be the main control centre of the brain, which in turn responds to various sensory stimuli and regulatory mechanisms. Input signals stimulate a satiety centre, where a certain output is given in the form of a response. Stimulated nerves that pass through the brain cause nerves to transmit information to other organs, i.e.: gizzard, liver, small intestine and pancreas. These input signals either come from the feed per se, in the form of visual or textural properties or are sensorial properties (colour, shape, smell). Birds are sensitive to visible properties and will not easily consume feed that they do not recognise or that seem oddly shaped. Birds go through an adaptation phase where they get used to the properties of feed. Signals are also created in the small intestine after feed was ingested (Ferket & Gernat, 2006).

d. Dietary factors

The following dietary factors or mechanisms are potential contributors to the regulation of feed intake (Gous, 2013):

- Nutrient composition of the formulated feed
- Raw materials included as well as the inclusion level of each
- Feed form (pellet, crumble or mash) and the quality thereof (Saleh *et al*., 2004)

There are several dietary factors that allegedly influence feed intake, especially if the dietary nutrient composition is either in great excess or deficient to the broiler's nutritional requirement. According to Ferket & Gernat (2006), meat-type breeds are less responsive to dietary influences when compared to layer-type birds, since they have been selected for BW and additive body weight gain over a long period of time. Therefore, these broiler breeds tend to consume to maximum gut fill if they are not limited or restricted by toxicities (dietary), environmental, managerial or disease factors.

e. Dietary energy

According to Ferket & Gernat (2006), the dietary energy content of feed has the most predictable outcome or effect on feed consumption in broilers. Basically, birds will attempt to consume enough feed to meet their metabolic energy requirement which is dependent on their energy requirements for body maintenance as well as growth, development and production. Inevitably, maintenance requirement is a priority over a certain level of development as well as production and is influenced by various factors, such as (Ferket & Gernat, 2006):

- General health status of the bird (exposure to diseases or immunity)
- Degree of mobility (determined by the stocking density and social interaction)
- Body heat loss (determined by the ambient temperature, relative humidity and air speed)

Therefore, in order to meet this requirement, feed intake will increase as the dietary energy content decrease. The next limitation that will control feed intake will either be from a filled gut or other physiological limits. However, according to Dozier *et al*. (2007), literature is inconsistent as to whether broilers have the ability to adjust caloric intake when the diets fed vary in energy content; or even to eat to a certain gut capacity regardless of the dietary apparent metabolisable energy (AME).

In the experiment conducted by Saleh *et al*. (2004), FCR at Day 21 was significantly improved as the average dietary energy level increased ($P < 0.05$) and demonstrated a quadratic response to the average dietary energy density by reaching a plateau at approximately 3267 ME kcal/kg (13.7 ME MJ/kg). FCR at Day 42, 49, 56 and 63 showed similar trends where a plateau was reached

at approximately 3304 ME kcal/kg (13.8 ME MJ/kg). These results demonstrated that the broilers consumed less feed at a certain dietary energy level and could innately consume lower amounts; possibly due to satiety mechanisms which allowed the birds to lower their feed intake at higher energy density and therefore resulted in a lower FCR.

f. Dietary protein and amino acids

Dietary protein and AA are known to have a more indirect effect on FI. Specific AA imbalances (excessive or deficient) have the ability to alter feed intake very rapidly. This is even possible in small chicks, suggesting that the subsequent effect, whether positive (improvement in growth) or negative (growth depression), is not the direct response to the imbalance. The mechanism(s) that control FI is therefore sensitive to certain AA concentrations or the balance between certain AA (Ferket & Gernat, 2006).

g. Dietary vitamins and minerals

Primarily, vitamins and minerals function as metabolic cofactors even though macro minerals such as calcium and phosphorus serve as structural components. Therefore, vitamins and minerals should only influence FI when dietary levels are either excessive or deficient several times above and below the nutritional requirements. Deficient dietary vitamin or mineral levels may cause a metabolic disorder which causes an indirect adverse effect on FI (Ferket & Gernat, 2006). However, slight mineral deficiencies may stimulate feed intake as the bird would consume more feed, attempting to reach the required level of the particular mineral. In contrast, excessive dietary vitamins and minerals result in possible refusal to consume the feed and are often associated with a significant increase in water intake. For example, dietary salt is used to stimulate intake, but in excessive levels it will depress FI and stimulate water consumption. Excess dietary calcium is also known to depress FI in growing and developing broilers (Ferket & Gernat, 2006).

h. Deficient diets

With reference to Emmans' (1981) proposition there are, however, controversial findings to broilers' reaction on marginally deficient feeds. Fast-growing broilers would sometimes select a diet which do not allow maximum growth i.e. nutrient deficient diet; and in order to compensate for the particular deficiency they would increase intake and consequently overeat on other

nutrients. Or this simply indicates that broilers cannot select an optimal diet.

Contradictory to this specific proposition, there is also evidence where broilers have been found to be able to select proportionally from two diets with differing energy or protein levels, in order to meet their varying requirements during growth (Nielsen, 2004). Another opposing outcome to Emmans' theory was seen in Macleod (1997) where heat production by means of respiration calorimetry was measured. There was some evidence for greater fasting heat production in growing chickens fed a lysine-deficient diet. Yet, most of the effects on energy utilisation efficiency were caused by the large (about 80%) decrease in feed intake. In actual fact, in most cases where a diet deficient in a single AA was fed, the major effects on energy retention was through a reduction in feed intake (Macleod, 1997).

i. Natural compounds

Natural occurring compounds or anti-nutritional factors such as phytates, protease inhibitors or even factors that are produced either as a result of fungal or microbial metabolism or by the plant *per se*, are all natural components that can have negative effects in the animal, consequently impairing the availability and absorption of the nutrients, depressing feed intake or reducing the growth of the animal. However, there are processing methods available to neutralise or detoxify such components (Ferket & Gernat, 2006).

j. Water consumption

Water is the most essential nutrient for a bird even though, the value in terms of requirement cannot necessarily be determined. Water consumption is easily twice that of feed intake and requirement is based on environmental temperature, humidity, diet composition, growth rate and the functionality of the broiler's kidneys, therefore, their ability for water resorption. Basic water: feed ratio is acknowledged across different feed forms: pellets, crumbles and mash and also serve as an indication of feed intake, since it should stay relatively the same. Water quality and the level of minerals are important, since excessive minerals could cause a decrease in water intake which could in turn results in a decrease in feed intake (Ferket & Gernat, 2006).

k. Management factors

The following factors or mechanisms are potential contributors to the regulation of feed intake (Ferket & Gernat, 2006; Gous, 2013):

- Environmental (temperature, wind, humidity) control
- Stocking density
- Disease challenges and control (vaccination programs and bio-security)
- Access to feed and water
- Temperature stress
- Immunological responses

2.2.2 *Ad Libitum* feed intake

The *ad libitum* intake of broilers is on average two to three times that of their maintenance requirements (Boekholt *et al*., 1994). Emmans' (1981) theory of feed intake suggests that a bird will attempt to consume sufficient amounts of a given feed to enable growth at its own genetic potential, thus allowing over consumption of energy if the feed is marginally deficient in an essential nutrient such as protein or a specific AA.

2.3 Major nutrients

2.3.1 Requirements

For many years it was assumed that chickens tend to eat to meet their energy needs, assuming that the diet is adequate in other essential nutrients (Saleh *et al*., 2004). Besides energy being one of the main driving nutrients behind formulations and production costs, recent nutrition research has focused vastly on the effect of different EI and PI, i.e. the slightly increasing or decreasing effect of dietary energy or protein concentrations relative to recommendations (Boekholt *et al.*, 1994).

Generally, dietary nutrients are in relationship to dietary energy, and by maintaining this natural relationship in a constant ratio, improved performance (growth rate and feed efficiency) is plausible with inclining levels of dietary energy, as long as amino acids are balanced (Dalibard & Paillard, 1995; Saleh *et al*., 2004). Even so, the AA balance has changed with an increase in the AA requirement at particular earlier growth periods and therefore a decreased requirement toward slaughter age (Dozier *et al*., 2006).

Farrell *et al.* (1973) concluded in their experiment that when broilers were kept under commercial conditions and given various diets with differing energy concentrations, there were large differences in their growth rate, FI, FCR and dressing percentage.

Thus, the amount of energy and protein (AA) that the broiler required, varies widely with modern genotypes and it is not clear whether these particular requirements are truly known (Ferguson *et al*., 1998).

2.3.2 Energy

a. Energy requirement

Commercial production and performance are mainly determined by environmental factors of which nutrition, especially energy, is most important of all, as it solely contributes approximately 65 to 70 % of production costs (Saleh *et al*., 2004). It is difficult to determine a basis on which dietary energy concentration can be recommended for a broiler chicken production since differences occur in the response of various broiler breeds and strains to diets with the same or different energy concentrations (Farrell, 1974).

At very low dietary energy concentrations in feeds, growing chicks may not meet its requirements. On the contrary, at high energy densities birds may consume excessive amounts of energy (more than required, even for maximum performance). The excess energy may then be deposited as fat, depending on its genetic potential to do so (Farrell *et al*., 1973). Farrell (1974) referred to Scott *et al*. (1969) who suggested that chickens do not adjust their energy intake exactly, but actually consume more feed as the energy concentration of the diet increases thus resulting in large amounts of fats deposited in the carcass (Farrell, 1974).

However, in the discussion of Farrell (1974) it was pointed out that there is an optimum energy concentration in the diet beyond which performance of birds did not appear to improve any further and even deteriorated in some cases. According to these results, at energy levels above approximately 3.3 Mcal ME/kg (13.8 MJ ME/kg) of diet, feed intake, energy intake, calories required per g of body weight gain and days to reached a particular live weight, generally increased; while the percentage of actual feed energy that was retained in the carcass, decreased. Growth rates of the broilers did not increase at energy levels above 3.1 Mcal ME/kg

(13.0 MJ ME/kg). The gradual improvement in performance of birds fed on diets that increased in dietary energy concentrations up to 3.3 Mcal ME/kg (13.8 MJ ME/kg) might have been due to an increasing portion of energy stored in body weight gain and therefore, less energy was used for maintenance as birds grew more rapidly.

In the experiment of Saleh *et al*. (2004), BW at day 21, 42 and 49 improved significantly as the dietary EL increased ($P < 0.05$) and peaked at an average dietary energy density of 3276 ME kcal/kg (13.7 ME MJ/kg). Above this 'peak' energy level, BW started to decline, but still had a higher BW than birds that received diets of less than 3276 ME kcal/kg (13.7 ME MJ/kg). The increase in BW was not as great as the increase in dietary nutrient density and appeared to diminish in magnitude of increase as the birds aged. The same trend was noticed by Farrell *et al.* (1973) as well as Farrell (1974) who reported the same reduction in performance at higher energy levels.

The results of the two trials conducted by Farrel *et al.* (1973) indicated that in order to achieve and maintain maximal growth rate and efficiency of conversion of ME to body weight gain, the dietary ME level had to range from 3.00 to 3.40 Mcal/kg (12.6 to 14.2 MJ/kg). Although this was higher than recommended by the ARC (1963), it was in agreement with the NRC (1971) recommendation of 3.20 Mcal/kg (13.4 MJ/kg). Farrel *et al.* (1973) did mention that it would be appropriate for Committees to recommend a minimum and maximum value when such recommendations were given; since a single value imposes a constraint on ration formulation which may not be biological or economical justified or feasible.

The energy requirement for maintenance was found to be affected by (Shalev & Pasternak, 1998):

- Environmental temperature
- Breed
- Differences in age
- Physical activity
- Feathering density
- Basal metabolic rate
- Surface area of bare skin
- Body temperature
- Body composition

b. Energy consumption

Renner (1964) presented the results of chicks fed high carbohydrate compared to chicks fed 'carbohydrate-free' diets with different feed protein contents for both feeds. Neither the level of protein nor the source of energy affected energy consumption and since the diets were of equal caloric density, the data indicated that equal amounts of feed were consumed. It was therefore concluded that chicks did not compensate for the reduced protein level (PL) of the diet by increasing their FI. This coincided with earlier results reported by Hill & Dansky (1954), adding to the concept that chicks eat to meet their energy requirement. These results do not support the concept proposed by Donaldson *et al*. (1957) where chicks increased their EI in an effort to obtain sufficient protein. The EI was relatively constant per chick for the period, although it was considerably higher in the protein-deficient groups relative to body size, which seemingly accounted for the increased fat deposition (Renner, 1964).

According to Saleh *et al*. (2004) improved growth rates are a result of genetic selection of broilers to consume feed at almost full capacity irrespective of the dietary energy levels. Therefore, at higher energy levels (EL), more rapid gains, elevated body weight-to-age and higher yielding meat produced over time, allow capital costs, such as housing, equipment and labour, to be reduced. Higher dietary EL may be economical if it provides a more rapid rate of gain and subsequently, a greater number of flocks per year. Even so, raw material costs as well as production costs of high energy diets may negate the benefits of enhanced performance.

In the experiment of Saleh *et al*. (2004) feed consumption tended to remain the same up to an average dietary energy level of 3148 ME kcal/kg (13.2 ME MJ/kg) at 42 days, after which the FI declined. The decline in FI was not proportional to changes in the average dietary EL and it was concluded that the modern broiler did not regulate FI by maintaining isocaloric consumption.

c. Energy retention

Apart from the required energy and AA levels, the physiological influence of EI on protein and fat retention, as well as actual BW and body weight gain, is beneficial to the understanding of nutrition (Boekholt *et al.*, 1994). Unfortunately, there is limited information available on how dietary energy is deposited during a broiler's short commercial life cycle. Established data demonstrates the influence of ME intake on the performance and body composition of developing broilers (Lopez &

Leeson, 2005). A trial conducted by Boekholt *et al*. (1994), showed that during the growth phase, broilers deposited feed nutrients efficiently into various body tissues, such as fat or protein (Boekholt *et al*., 1994; Lopez & Leeson, 2005). Therefore, ME intake is usually partitioned effectively into ER, as protein or fat, and heat production as stated in Eq 1 and Eq 2 below (Lopez & Leeson, 2005):

Equation 1

ME = HP + ER

Where: ME = Metabolisable Energy; HP = Heat Production; ER = Energy Retention

Equation 2

ER = ERP + ERF

Where: ER = Energy Retention; ERP = Energy Retained as Protein; ERF = Energy Retained as Fat

Under thermoneutral conditions, HP represents heat that is associated with the utilisation of ME intake for maintenance (MEm) and production. In juvenile or growing broilers, the heat utilised for production only represents approximately 52 to 64% of intake. Therefore, ER = ME – HP and when evaluating ER, it is necessary to take into consideration the efficiency of utilisation of ERF (kf) and ERP (kp) (Lopez & Leeson, 2005).

Boekholt *et al*. (1994) reported a similar partitioning as Lopez & Leeson (2005), but took the ME intake into account as the actual weight gained by the bird, thus the relation between weight gain and retention of protein and fat was assessed with the following regression equation:

Equation 3

WG = a + (b x P) + (c x F)

Where: WG = weight gain (g per animal per day); P = protein retention (g per animal per day); F = fat retention (g per animal per day)

From the results of the study by Boekholt *et al*. (1994), a reduction in dietary energy supply, with associated reduced ME intake, produced a clear effect on body weight gain and ER (as ERP and ERF). For example, a daily reduction of 27% in ME intake resulted in a reduction of 25% protein retention and 69% in fat retention; and an additional reduction of 11% ME intake, influenced the fat retention by a further 32% with minor fat mobilisation, but did not influence protein retention noticeably, as presented in Figure 2-1 (Boekholt *et al.,* 1994).

When considering the relation between ERP and ERF a graphical display (Figure 2-2) would prove to be linear at all values of EI. Boekholt *et al*. (1994) proposed that by increasing the energy supply (i.e. higher density diets) excessive energy would be retained, but in a remaining constant ratio between protein and fat. Values deducted from Figure 2-2 showed that at an ER of 179 kJ/ Metabolic Weight (MW)/ day, the ER as fat is zero and hence only protein is retained and known as “primer growth of protein.” However, at an ER lower than 179 kJ/ MW day, protein is still retained, but at the expense of fat mobilisation; thus confirms that at low EI, there is a high preference for the retention of protein, even if it results in the mobilisation of body fat (Boekholt *et al.*, 1994).

Furthermore, if energy supply is increased beyond 179 kJ/day, additional amounts of protein and fat are retained and the approximate constant ratio displays energy retained as fat at 85% and therefore energy retained as protein is 15%. Therefore, at higher EI relatively more energy will be retained in fat and less thereof in protein.

For example, with an inclining energy contribution at increments of ca. 100 kJ, energy retained consists of 85 kJ fat and of 15 kJ protein. From this particular experiment it could be concluded that extra fat and protein were retained in an energy ratio of 85: 15 and each additional unit of gain is composed of similar amounts of protein and fat. This relationship holds for other young growing animals, even though the ratio may differ. From the equation, proportional weight gain per gram of retained protein and fat was estimated at 3.8 g and 1.1 g, respectively (Boekholt *et al*., 1994).

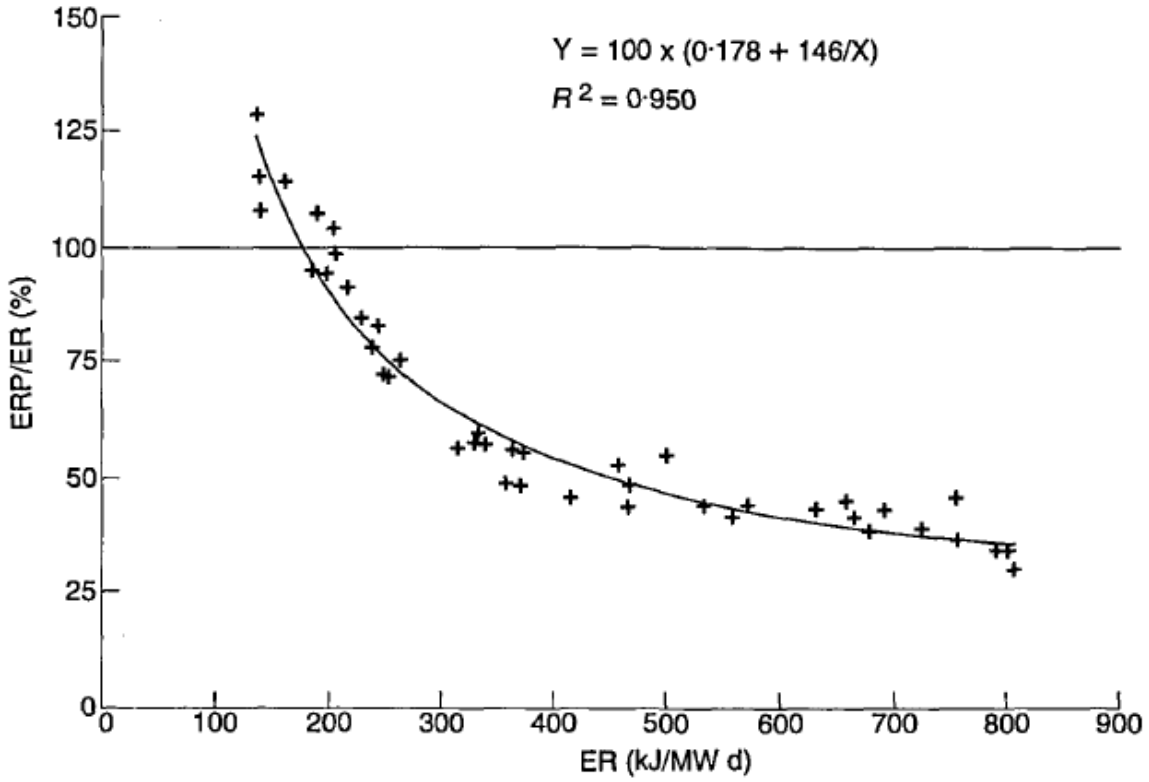

Figure 2-1 Energy retention in protein (ERP) as a percentage of energy retention (ER) in relation to energy retention (Boekholt *et al.*, 1994)

However, if the total amount of protein retained is expressed as a fraction of the total energy retained (ERP/ER as %), the contribution of protein to energy retention changes significantly. As energy supply and energy retention increases, this fraction decreases gradually to approximately 35% at maximal growth (Boekholt *et al.,* 1994).

Apart from the original results of 179 and 170 kJ/ MW/day in Boekholt *et al*. (1994), results from an additional diet were revealed; where at zero fat retention, the protein retention was 221 and 201 kJ/MW/day respectively. Both diets showed a propensity for protein at lower EI. The results concluded that if protein was not the first limiting nutrient, higher energy supply in growing broilers not only resulted in protein retention but also in increased fat retention; and incremental levels of dietary energy cannot stimulate protein retention if protein is limiting.

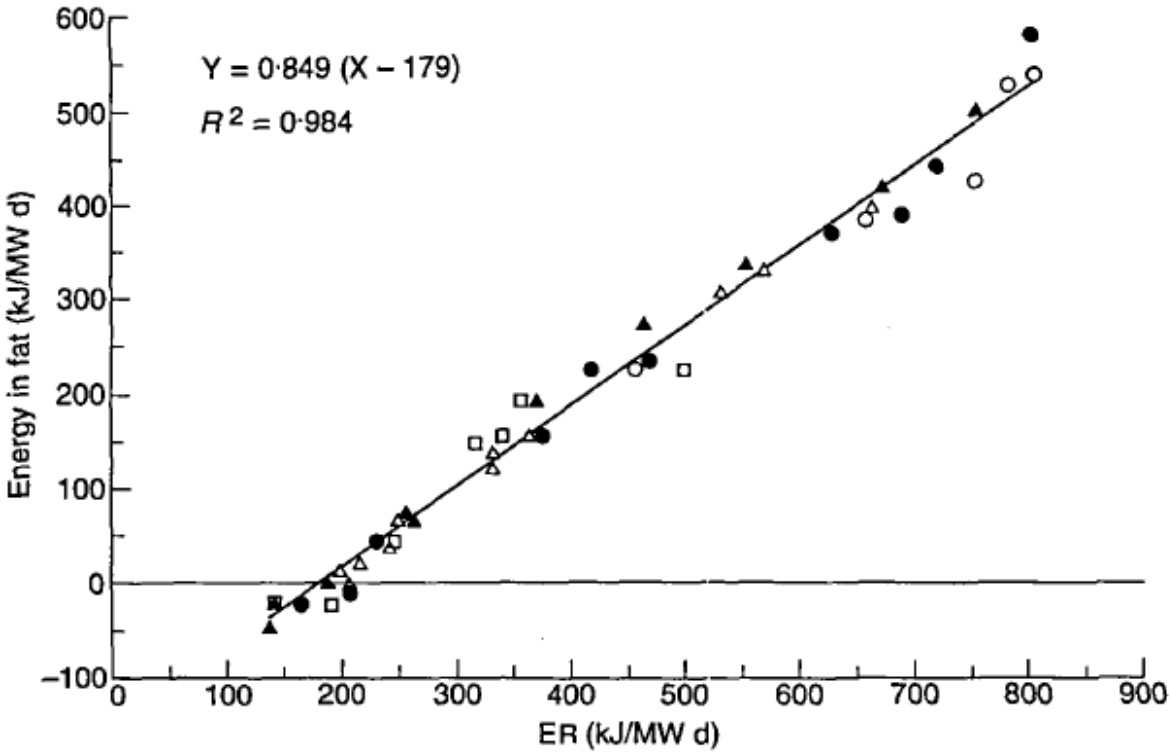

Figure 2-2 Relationship between energy retained as fat and energy retention (ER). Both are expressed per metabolic weight ($W^{0.75}$) (Boekholt *et al*., 1994)

d. Efficiency of utilisation of metabolisable energy

Energetic efficiency of ME use for tissue gain is dependent on numerous variables. It has been proved that actual efficiency vary with each substrate source for lipogenesis at approximately 84%, 75% and 61% for fats, carbohydrates and protein, respectively. However, the high availability of fat ME for tissue gain is dependent on the fat used for lipogenesis *per se* (Boekholt *et al.,* 1994).

In order to gain energy for tissue muscle from the utilisation of protein, the process is dependent on the biological value (BV) of the protein source and acknowledgement that the BV is not constant among sources. Therefore, one could summarise that a bird's energetic utilisation efficiency for any substrate is the net result of partitioning consumed substrate energy into maintenance as well as subsequent protein and fat accretion (Teeter *et al*., 1996).

In Table 2-1, Saleh *et al*. (2004) displayed the effects of dietary nutrient density on the absolute and relative BW of male broilers. A decrease in body weight gain was noticed at levels higher

than the average dietary EL. This was coupled with a moderately constant FI which, in turn, resulted in a constant energy utilisation (ME kcal/kg of gain) up to an average dietary EL of approximately 3267 ME kcal/kg (13.7 ME MJ/kg). At all ages, with the exception of day 56, the broilers were significantly less efficient in utilising the EI at higher average dietary energy levels. Waldroup *et al*. (1976) reported similar results, where energy conversion was essentially equal from 2970 to 3300 ME kcal/kg (12.4 to 13.8 ME MJ/kg), with a decline in energy utilisation when the dietary EL exceeded 3300 ME kcal/kg (13.8 ME MJ/kg).

Table 2-1 The effect of dietary nutrient density on absolute and relative body weight of male broilers (Saleh *et al*., 2004)

Mean dietary energy (ME kcal/kg) [1]	Poultry Oil (%)	Body Weight (g)					Body weight (Relative)					
		21d	42d	49d	56d	63d	Relative energy content[2]	21d	42d	49d	56d	63d
3023	0	681^{d}	2119^{c}	2652^{e}	3149	3625^{b}	100.0	100.0	100.0	100.0	100.0	100.0
3069	1	692^{cd}	2128^{bc}	2679de	3132	3604^{b}	101.5	101.6	100.4	101.0	99.5	99.4
3109	2	718^{ab}	2179^{abc}	2702^{cde}	3194	3651^{b}	102.8	105.4	102.8	101.9	101.4	100.7
3148	3	713^{bc}	2158^{abc}	2698^{cde}	3170	3664^{ab}	104.1	104.7	101.8	101.7	100.7	101.0
3188	4	726^{ab}	2187^{abc}	2757^{abc}	3222	3738^{ab}	105.5	106.6	103.2	103.9	102.3	103.1
3227	5	708^{bc}	2201^{ab}	2720^{bcde}	3156	3717^{ab}	106.7	103.9	103.8	102.5	100.2	102.5
3267	6	740^{a}	2224^{a}	2816^{a}	3277	3778^{a}	108.1	108.7	104.9	106.1	104.1	104.2
3304	7	722^{ab}	2204^{a}	2780^{ab}	3248	3736^{ab}	109.3	106.0	104.0	104.8	103.1	103.1
3344	8	724^{ab}	2208	2741^{bcd}	3185	3624^{b}	110.6	106.3	104.2	103.3	101.1	99.9
3383	9	729^{ab}	2200^{ab}	2727^{bcd}	3207	3628^{b}	111.9	107.0	103.8	102.8	101.8	100.1
F-Prob			0.0009	0.05	0.0005	0.27	0.007					
SEM			8	24	23	38	41					

[1]Mean dietary energy level (ME kcal/ kg) of diets fed 0 to 21d, 21 to 42d, and 42 to 63d.

[2]Diet energy relative to treatments with no added poultry oil.

^{abcde} Means in column with common superscript do not differ significantly (P<0.05)

According to the experiment by Farrell (1974), there was a noticeable improvement in the efficiency of utilisation of ME between two specific diets when determined by the percentage ER in the tissue. The two diets were between the EL of 2.7 Mcal/kg and 3.2 Mcal/kg (11.3 MJ/kg and 13.4 MJ/kg). However, above the range of 3.2 Mcal/kg (13.4 MJ/kg) there was no apparent accelerated change in growth rate as seen before. The reduction in energy efficiency as broilers age, possibly suggest that the birds' ability to utilise supplemental fats increase with age (Saleh *et al*., 2004).

2.3.3 Protein

a. Protein requirements

Dietary protein quality is an important factor influencing the efficiency of protein utilization (Urdaneta-Rincon & Leeson, 2004). Eits *et al*. (2003) defined the requirement for protein as the minimum level of protein in the diet at which some measure of output is optimised, for example, weight gain. A broiler's response to dietary CP is thought to be influenced by the level of the first limiting amino acid. Conversely, Urdaneta-Rincon & Leeson (2004) stated that the dietary CP influenced the AA requirements; where the lysine requirement, as a percentage of the diet, increased linearly with the dietary CP level. The order of requirements for protein can be listed from high to low priority, in measurement of response to CP levels (Pesti, 2009) namely:

1. Live body weight
2. Maximal feed utilisation efficiency
3. Maximum carcass lean weight and
4. Minimal carcass fat

Genetic differences among genetic lines contribute to the response of chicks to varying dietary levels of CP and ultimately, the requirement of AA. Broiler responses of economic interest such as daily weight gain, FCR and breast meat yield are known to be optimised by increasing AA concentrations, improving the AA balance, or even both (Vieira & Angel, 2012). Therefore, even though CP is required in a particular priority, the required level of lysine to maximise specific performance traits differs accordingly (Ferguson *et al*., 1998; Sterling *et al*., 2006; Vieira & Angel, 2012):

1. Highest requirement for minimising abdominal fat pad percentage
2. Followed by minimising the FCR and
3. Maximising breast meat yield and body weight gain

A maximum performance response is reached when the response plateaus, whereas an optimal response provides the highest return per input. Therefore, linear feed formulation strategies differ in how dietary AA are included and understanding how these strategies affect the broiler responses could be beneficial (Vieira & Angel, 2012). A similar response was noticed in the study conducted by Eits *et al*. (2003), detecting that the response of growing broilers to dietary PL was characterised by declining increments of response as the PL in the diet increased; up to a level

where a plateau in output was reached. An absolute growth rate (mg/d) reached a plateau in the study by Urdaneta-Rincon & Leeson (2004) when dietary lysine levels higher than 1.22% and CP levels greater or equal than 210 g/kg of feed were provided. The CP and lysine requirement for optimal growth was achieved and the lack in response in increased muscle protein deposition indicated the maximum predetermined genetic potential for growth had been reached (Urdaneta-Rincon & Leeson, 2004).

As discussed earlier, a selection experiment done by Tesserand *et al*. (2003), where chickens of so-called “quality” line (QL) were selected for high breast meat, low abdominal fat percentage and as high body weight as possible; aimed at improving broiler carcass quality in terms of body composition. The QL was compared to a control line (CL) in order to facilitate comparison between the lines and to estimate the genetic gain of each generation. Researchers stated that although growth was controlled by a complex interaction of genetics, hormonal and nutritional factors, dietary protein also has a powerful regulatory influence on muscle growth and protein turnover. From the results the differences between the QL and CL chickens could clearly be observed (Table 2-2). Factors such as weight gain, feed intake and FCR were noticeably different between the chickens fed the HP diet and the LP diets, as well as between QL and CL chickens.

Common nutritional practice is to reduce protein and increase the energy of diets as bird age (Mendonca & Jensen, 1989; Nielsen, 2004; Lopez *et al*., 2007); this is due to the declining protein gain as a percentage of total body weight gain and increased feed intake (Corzo *et al.,* 2010). Consequently, requirements (as % of diet) for all AA decrease with age and weight (Baker, 2008). Higher AA diets during early stages are economically advantageous; since these diets can fulfil the young broiler’s high requirement for AA, despite a low feed intake (Kidd *et al*., 2004).

The ratio of AA in muscle and other body tissues are constant regardless of growth rate (Corzo *et al*., 2010). As dietary PL increase, fat deposition in the body decrease (Mendonca & Jensen, 1989) together with an increase in body protein, carcass yield (Pesti, 2009) and moisture. Extensive studies with lysine have also shown that for maximal feed efficiency, a higher dietary level of lysine than maximal body gain is required (Baker, 2008). Therefore, finisher diets with protein levels in excess of the requirement for optimum growth could be used to lower fat content in the carcass at market age (Mendonca & Jensen, 1989).

Table 2-2 Growth performance of quality (QL) and control (CL) chickens fed a single diet differing in protein content from 21 days of age (Tesserand *et al*., 2003)

	QL		CL			Statistical analysis (P-values)		
Item	**HP diet[2]**	**LP diet**	**HP diet**	**LP diet**	**Pooled SEM**	**Diet Effect**	**Line Effect**	**Interaction**
21-d body weight, g	710	650	650	654	7	NS	<0.001	NS
33d body weight, g	1524	1366	1366	1270	21	<0.001	<0.001	NS
Daily BW gain, g/d								
28 to 33d	75.5	63.7	67.7	60.2	1.8	<0.001	<0.001	NS
21 to 33d	67.8	54.7	59.6	51.4	1.4	<0.001	<0.001	NS
daily Feed intake, g/d								
28 to 33d	129.4	137.5	120.8	129	3	<0.001	<0.001	NS
21 to 33d	112.5	119.4	103.4	111.2	2.3	<0.001	<0.001	NS
Daily Protein intake, g/d								
28 to 33d	27.92	16.71	26.06	15.67	0.48	<0.001	<0.001	NS
21 to 33d	24.28	14.5	22.31	13.61	0.36	<0.001	<0.001	NS
Feed: gain, g:g								
28 to 33d	1.717	2.166	1.789	2.158	0.031	<0.001	NS	NS
21 to 33d	1.661	2.192	1.737	2.177	0.026	<0.001	NS	NS

[1]Values are means of 15 chickens per diet x line combination

[2]HP = high protein diet (215.8g CP/kg) ; LP = low protein diet (121.5g CP/kg)

From the study done by Eits *et al*. (2003), dietary history may be particularly relevant when diets low in protein were fed during the early developing phases. Such feeding strategies have even showed a possible improvement in the resistance of broilers to metabolic disorders, such as ascites, in later stages. Broilers given a LP diet at an early age showed to compensate in body weight gain during a later stage, such as the re-alimentation period. This potential compensatory growth had a higher probability for males than females and may have even been less within fast growing strains compared to slower growing strains. As a result, even feed efficiency improved after an early protein restriction, most likely due to a lower fat content in body weight gain. In addition, the growth curve became more convex and therefore, in theory, maintenance requirements lowered. The total PI required to reach a specific BW may be reduced in this particular feeding strategy, thereby creating a protein sparing effect that has advantages for both production costs as well as environmental nitrogen losses (Eits *et al*., 2003).

Scheuermann *et al*. (2003) also observed a higher point of inflection on the Gompertz growth curve for breast weight compared to BW; at higher dietary AA levels. These dietary AA levels are reported to no longer improve BW, but continue to increase breast muscle deposition, therefore improved body yield.

Berhe & Gous (2008) pointed out that there is only circumstantial evidence that some strains show variability in production traits such as BW, when high or low protein diets are fed. It is more

apparent that strains of broilers as well as individuals within a strain, over consume feeds with marginal deficiencies of which the limiting nutrient is a major nutrient such as energy or protein.

b. Protein accretion

Protein accretion is usually predetermined by the bird's genetic potential, assuming the diet supplies adequate amounts of balanced AA (Lopez *et al*., 2007). The quality and quantity of dietary CP are important factors and influence the efficiency of protein utilisation for performance, muscle protein mass, protein metabolism and muscle growth. As stated earlier, if a young broiler's dietary lysine requirements increase linearly with the increase of CP, protein synthesis would probably increase as dietary lysine increases in higher CP diets (Figure 2-3). However, the NRC (1994) stated the dietary lysine density would be beyond normal recommendations. Even so, the maximum protein deposition and growth rate would be associated with an optimum requirement of CP and AA; therefore AA levels above those needed for optimum growth does not improve protein accretion, likely due to increased rate of protein catabolism (Urdaneta-Rincon & Leeson, 2004).

It was confirmed earlier that as broilers grow, they deposit feed nutrients (energy) in body tissues, mainly as protein and fat. From this energy deposit, it was suggested that an improvement in the carcass composition would be better achieved by manipulation of carcass fat rather than protein (Boekholt *et al.*, 1994). Even so, this relationship between the deposit of fat or protein is the result of the interactions between (Lopez *et al*., 2007):

- Nutrition
- Sex
- Genotype (bird strain)
- Environmental conditions

Muscle mass is controlled by the number and size of myofibres and this number is fixed during embryonic development (Urdaneta-Rincon & Leeson, 2004). Muscle accretion therefore depends on the balance between synthesis and degradation of muscle protein (Tesseraud *et al*., 2000); which is a more complex procedure since muscle protein consist of intertwined fibres, whereas deposited fat is individual cells of which water and energy are the main constituents (Pym & Farrel, 1977).

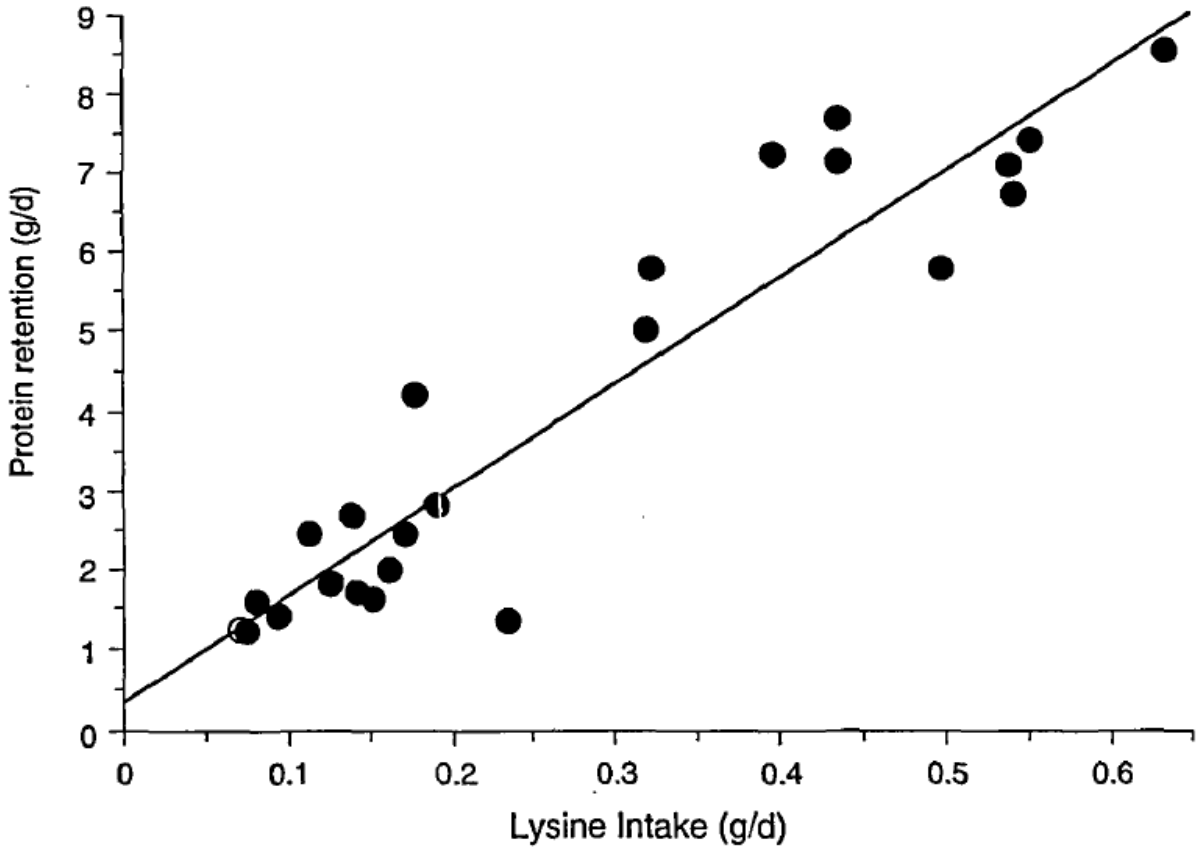

Figure 2-3 The relationship between protein retention and lysine intake (Macleod, 1997)

Despite this, the rate of protein synthesis and proteolysis are functions of protein and lysine intake, therefore higher dietary intakes increase the protein synthesis and degradative capacities of muscle cells (Urdaneta-Rincon & Leeson, 2004).

As BW increases, the accretion and quantity of body protein as well as the deposition of fat occur at different rates and efficiencies; with fat depositing at potentially higher rates with age (Lopez *et al*., 2007). The differences in potential rates of protein accretion is not solely caused by variation in efficiency of utilising dietary protein; higher daily requirement for protein and energy will also be needed to maintain higher growth rates (Morris & Njuru, 1990).

Changes in the proportion of retained protein and fat are important in terms of carcass composition and can be manipulated into efficient processes and ultimately into the desired final product.

Increased dietary energy causes higher fat retention in broilers, although the fat was mainly associated with abdominal organs compared to edible parts (Boekholt *et al*., 1994).

Alternatively, the use of variable dense diets with respect to AA, causes a response in protein accretion in the form of breast meat yield. Dozier *et al*. (2006) reported an improvement in growth performance and meat yield with the use of diets higher in nutrient density compared to the NRC (1994) recommendations. By invigorating diets with a higher proportion of AA from day 1 to 35 was found to be advantageous with respect to meat yield when the product demanded was heavy-weighted marketable broilers (Dozier *et al*., 2006). A reported increase in breast meat was noticed when broilers were fed diets formulated to 115% of lysine recommendation (NRC, 1994) from day 1 to 18 and 125% of lysine recommendation (NRC, 1994) from 19 to 42 days of age. Feeding high nutrient dense diets throughout the broiler's production cycle may, however, not be economical advantageous for live production costs, but may be optional for greater meat yield (Dozier *et al*., 2006).

c. Essential and non-essential amino acids

Dietary protein is used by broilers for many functions such as protein synthesis in the form of muscle accretion. AA are the building blocks of protein and are required to physically build muscle protein. Poultry species require a specific quantity and balance of dietary EAA and sufficient nitrogen for the synthesis of NEAA (Urdaneta-Rincon & Leeson, 2004). Individual EAA requirements are functions of the total CP level and by increasing the total CP level, whilst maintaining ideal ratios of EAA, increases performance. Consequently, AA requirements are proportional to CP content of a diet, therefore they are required in proportion to one another (or in proportion to EAA and NEAA) to synthesise body proteins (Pesti, 2009). This has been referred to as the 'ideal protein (amino acid) concept', forming the basis for the expression that AA requirements are in ratio to lysine (Baker, 2008; Pesti, 2009).

Factors affecting the ideal ratio are (Baker, 2008):

- Period of growth
- Sex
- Response to nutrients

Whereas the genetic potential of protein accretion and dietary energy density hardly has an effect on ideal ratios (Baker, 2008); and according to Mack *et al.* (1999), the ideal AA ratio should be based on digestible AA.

Amino acid requirements *per se*, are influenced by interactions between (Mendonca & Jensen, 1989):

- Dietary
- Environmental
- Gender
- Chronological
- Genetic factors

With genetic improvements, broilers consume less feed per unit of BW gain; hence, dietary AA needs of modern broilers should be increased compared with AA minimums used with broiler in previous years (Dozier *et al*., 2008). However, even if AA requirements change, the ideal ratio of EAA to lysine will only be affected marginally within a time period. Thus, the benefit of applying the ideal protein concept to formulation is that once an ideal ratio is established for a certain growth period, one can determine the lysine requirement under various environmental conditions as well as the requirements for all other AA (Mack *et al*., 1999). Ultimately, by identifying the AA profile, a formulated diet can be matched as close as possible to the required profile of a broiler at a certain body size and age in order for maximising performance (Mack *et al*., 1999).

AA requirement changes with body size and the AA profile for maintenance and growth differ and will continually changes during the growth period. Environmental circumstances will always be a contributing factor to the change in AA requirement; it is therefore evident that under ever-changing conditions over time, the ideal AA ratio would be an approximation of the optimum AA ratio required by the bird. Ultimately, it would be beneficial to feed a number of different diets from hatching to slaughter (Mack *et al*., 1999).

According to Fatufe *et al*. (2004) an attempt to model the requirements for EAA of livestock depends on the knowledge of individual components of the requirements as well as the factors that influences these components. For example, in growing animals, the main components of the quantitative EAA requirements are (Fatufe *et al*., 2004):

- Maintenance
- Protein accretion
- The EAA pattern in accreted protein and
- The efficiency of utilisation of individual EAA

d. Limiting amino acids

The birds' response to dietary CP is thought to be influenced by the level of the first limiting amino acid (Vazquez & Pest, 1997). Lysine is usually considered as the second limiting AA in broiler feeds and on going studies are conducted to determine the requirements for broilers (Urdaneta-Rincon & Leeson, 2004).

In Macleod (1997) the implied hypothesis was that "when protein synthesis becomes limited by the first-limiting EAA, amino acids present in excess of the resulting requirement enter the pool of substrates available as energy sources." Macleod (1997) also stated a strong negative correlation between Lys: CP and protein retention (as per gram of lysine intake), which may have resulted from catabolism of lysine. However, lysine had to be present in greater quantities relative to other AA. This particular negative correlation suggests that the reaction to the limiting AA, was actually improved by the presence of a tremendous amount of other AA. Compared to the severely deficient lysine experiment, Macleod (1997) mentioned that over a range of marginally imbalanced diets, he found no adverse effects of protein quality on lysine utilisation and even detected some implication of increased net utilisation with decreasing protein quality.

e. Determining the first limiting amino acid and its requirements

The quality of dietary protein is an important factor influencing the efficiency of protein use for performance; and the birds' response to dietary CP is thought to be influenced by the level of the first limiting AA. Dietary CP influences the AA requirements in broilers, therefore lysine requirements, expressed as a percentage of the diet, increase linearly with the dietary CP level (Urdaneta-Rincon & Leeson, 2004).

Most often, research estimates of lysine requirements have been based on multiple-range test analyses without any indication of the confidence in the estimate results. Alternatively, non-linear regression fitting techniques can be used in order to evaluate data and estimate the lysine

requirement and its confidence interval. However, due to large variation in broiler responses between experiments, it is a challenge to combine data for statistical analyses without making some alteration to the recorded data (Vazquez & Pest, 1997).

Attempting to model the EAA requirements depends on the understanding of individual components of the requirements as well as the factors that influences these components.

The main components of quantitative EAA requirements are (Vazquez & Pest, 1997):

- Maintenance
- Protein accretion
- EAA pattern in accreted protein
- The efficiency of utilisation of individual EAA

When considering additional variables, the following are important as well (Vazquez & Pest, 1997):

- Dietary concentrations
- Feed intake
- Gain to feed ratio

In modelling AA requirements the lack of data, which describes the efficiency of utilisation for the first limiting EAA, is a major weakness. Even so, many studies concerning the response of broiler chicks to variable dietary lysine concentrations have been published. An experiment with pigs and rainbow trout, suggested that the response to incremental lysine intake was non-linear and that it is diminishing at a level below the estimated requirement for high protein gain. Nonetheless, it is commonly assumed that efficiency of EAA utilisation for growth does not differ between genotypes within one species (Fatufe *et al*., 2004).

In the study conducted by Vazquez & Pest (1997), the researchers used two non-linear models to estimate the lysine requirements. Figure 2-4 displays the requirements based on the shapes of the “Ascending line with plateau” (ALP) and “Ascending quadratic with plateau” (AQP) response curves. Between the two models, the AQP model gave a higher lysine requirement estimate than the ALP model, due to the 95% confidence interval for percentage lysine requirement being wider in the AQP model; thus the model predicted a higher maximum response. This wider interval was however attributed to the nature of the AQP model. There was also a smooth transition from

ascending line to plateau noticed in the AQP model, compared to the abrupt change in the ALP model. The AQP model simply fitted the data slightly better than the ALP model for body gain, since it had a slightly higher R^2. Compare to gain, the opposite was observed for feed efficiency (the ALP model gave a slightly higher R^2 value).

As previously mentioned by Morris (1983), the ALP model always lead to false deductions about the optimum input. The slope of the ALP is constant on the ascending line and changes to zero at a break point; the model was therefore considered unsuitable to describe a response. The model assumed the same efficiency throughout the ascending part of the line, with a sudden change to zero efficiency, displaying difficulty to relate values to economics based on diminishing returns and efficiency. In comparison, the slope of the AQP model changed along the ascending quadratic line and this permitted a more sensitive adjustment of nutrient levels. Subsequently, it could indicate whether it is better to feed less or more lysine based on relative efficiency at different feeding levels and prices. A model that allowed such flexibility seemed more promising since economics is the driving force in poultry production (Vazquez & Pest, 1997).

f. Excess protein and amino acids

In the experiment of Macleod (1997), results proved that the large differences in CP: True Metabolic Energy (TME) can be accommodated without any indication of regulatory diet-induced thermogenesis. These differences in energy output could mainly be explained by differences in the amount and cost of protein accretion and fat deposition. Protein accretion was strongly correlated to heat production as well as to lysine intake (not total protein intake). Evidently, heat production on a lysine-limited, imbalanced AA mixture was no greater than on a balanced mixture with equal lysine concentrations. Even so, there was no indication of heat production stimulated by excess AA.

The results further confirmed the overruling quantitative importance of the cost of protein accretion relative to that of nitrogen excretion. The effects of imbalanced AA on heat production is small; the reduced costs of protein accretion may be a counter-balancing mechanism to the additional cost of nitrogen excretion (Macleod, 1997).

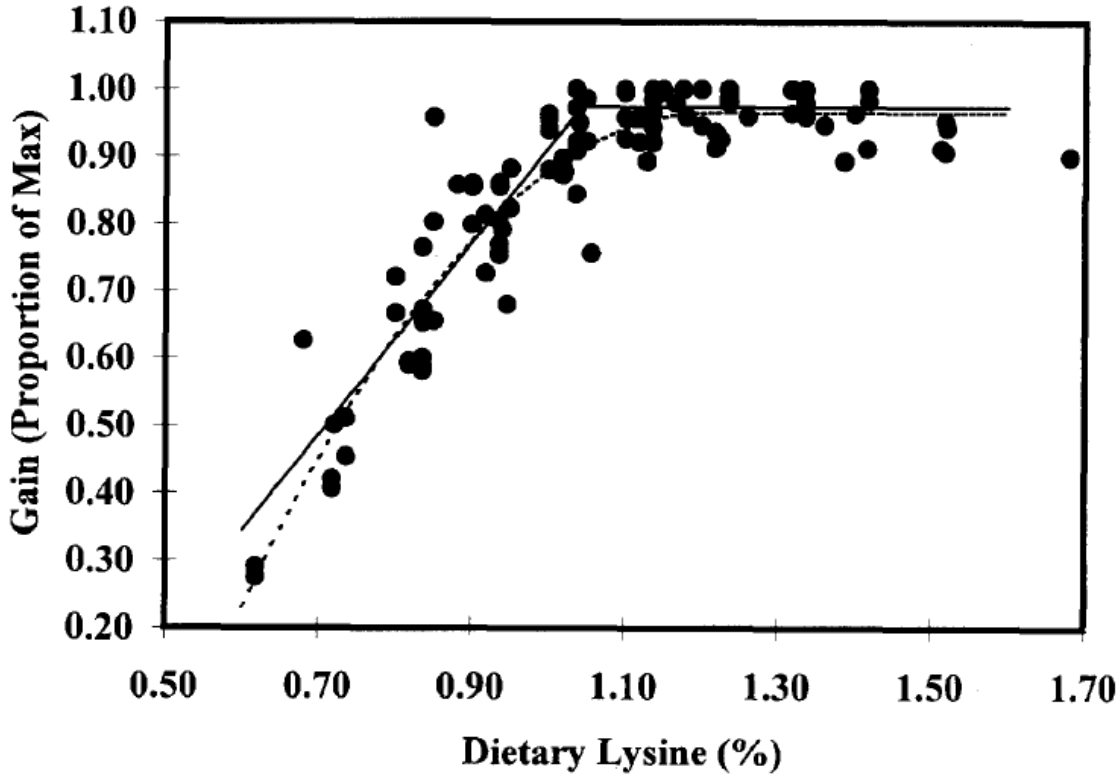

Figure 2-4 Results of the non-linear regression analyses showing the ascending line with plateau and ascending quadratic with plateau models (Vazquez & Pest, 1997)

The energetic cost of protein accretion in broilers has been estimated to be 0.7 kcal/ g (0.003 MJ/g) of protein synthesised (Urdaneta-Rincon & Leeson, 2004). When comparing the energy cost of protein synthesis and nitrogen excretion in molecular value, a quantitative comparison confirmed the energy waste of excess dietary protein (Macleod, 1997). The energy cost of incorporating an AA molecule into a protein, by the use of a peptide bond, is approximately 4 to 7 mol of ATP; in contrast to proteolysis at a cost of 1 to 2 mol of ATP for peptide breakdown (Urdaneta-Rincon & Leeson, 2004). The excretion of nitrogen, as uric acid in the faeces, resulting from the catabolism of AA used 6 mol of ATP/g/atom nitrogen. Therefore, the excretion of a single AA costs 6 mol ATP/mol AA for most AA containing 1 nitrogen atom, but as much as 18 mol ATP for histidine, which contains 3 nitrogen atoms (Macleod, 1997).

g. Synthetic amino acid supplementation

In order to reduce the excretion of nitrogen as well as the difficulty of filling a complete broiler diet with sufficient required AA, the partial substitution of whole protein with that of crystalline AA was implemented. Through this replacement, excessive dietary AA can be minimised in relation to their requirement, thereby bringing the dietary protein closer to the ideal protein and, in turn, decrease the dietary CP content (Macleod, 1997).

From an experiment in pigs, it was established that for each percentage point decrease in the dietary CP content, the amount of excreted nitrogen was reduced by 8%. In order to reduce the dietary CP, crystalline AA was used. However, in similar experiments with broiler chicks, growth performance and carcass composition became inferior to the group of broiler chicks fed standard high CP diets when the dietary CP content was lowered by more than 3 to 4 % points. The adverse effects occurred in spite of the low CP diets meeting the established AA requirements and even though they had optimal ratios of EAA to NEAA. Therefore, it is generally not advised to lower the dietary CP content by more than approximately 3 percentage points (Bregendahl *et al*., 2002).

With the establishment of the successful supplementation with synthetic AA to avoid increasing the total protein concentration of diets, a major consequence of supplementation is greater fat deposition in commercial broilers. There are two possible reasons for this: firstly, due to lower surplus protein to be metabolised and secondly, a greater proportion of dietary energy is consumed as carbohydrate and or fat; which in turn, is then deposited more effectively and efficiently as body fat (Macleod, 1997).

Waguespack *et al*. (2009) found that 0.25% L-Lys.HCl can be supplemented in a maize-soybean meal diet for broilers, without negatively affecting growth performance. But, the lysine supplementation was only successful if accompanied by supplemental DL-Met, L-Thr and Gly. The supplemental amounts of AA were based on a total lysine specification required by the broiler strain used in the experiment and comprised of the L-Lys.HCl as well as lysine provided by the maize-soybean meal diet.

2.3.4 Dietary protein to energy ratio

A hypothesis of Eits *et al*. (2003) was that if a LP diet is used in early growth phases, the performance level will be increased in later stages. However, the likelihood of any nutritional carry-over effect would depend on the sex of the bird also considering that the modern fast growing breeds have limited ability for compensatory growth. Considering the hypothesis, the experimental results indicated that broiler responses to dietary balanced protein level in later grower phases depend on the balanced protein level in the diet fed in earlier stages. These carry-over effects were found to be more prominent with growth rate and FCR, and to a lesser extent with average gain.

2.3.5 Dietary fat

It has been shown that the supplementation of dietary fat improve FCR and decrease FI (Dozier *et al*., 2007); and if the dietary nutrient balance is maintained, growing chicks can tolerate high levels of dietary fat (Renner, 1964). Fat can even completely replace carbohydrate in the diet of the chick without affecting growth rate. Carbohydrates are required for essential physiological functions, such as (Renner, 1964):

- Maintaining the blood glucose level
- Fatty acid oxidation
- The formation of various body constituents

Renner (1964) showed a significant interaction between dietary protein level and the energy source. Isocaloric substitution of fat for carbohydrate decreased growth when the diet contained 15.4 kcal/g (0.064 MJ/g) protein and increased when the diet contained 19.8 kcal/g (0.083 MJ/g) protein (Renner, 1964).

Similarly, chicks fed ‘carbohydrate-free’ diets containing 15.4 kcal/g (0.064 MJ/g) protein, of which non-protein energy was supplied by oil (soybean), grew at least as rapidly as chicks fed similar diets containing glucose. The results showed when measuring growth rate, the protein requirement of chicks fed ‘carbohydrate-free’ diet is no different from chicks fed diets containing carbohydrates; also that the chick had the ability to utilise large quantities of fat. ANOVA on the protein retention data indicated that the response to the level of protein in the diet was significant ($P < 0.01$), but the response to the source of energy was not.

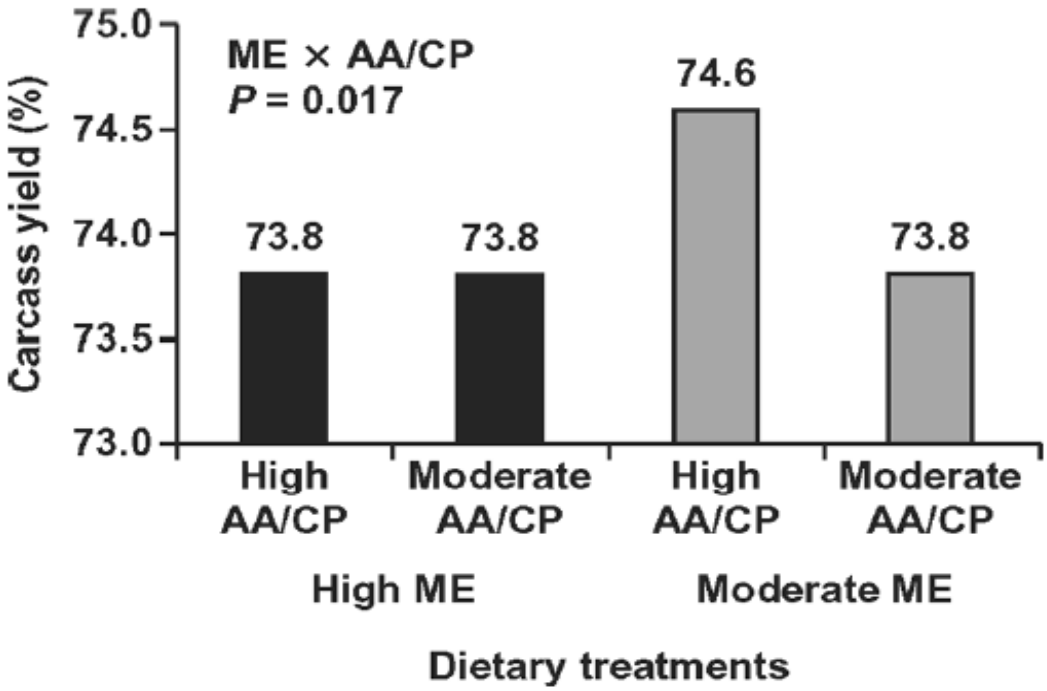

Figure 2-5 Carcass yield of male and female broilers provided diets varying in amino acid and apparent metabolizable energy density (Dozier *et al*., 2007)

It was concluded that the carbohydrate requirement of the chick can be met without diverting AAs from protein to carbohydrate synthesis, since chicks fed 'carbohydrate-free' diets utilised protein just as efficiently as chicks fed diets containing carbohydrate (Renner, 1964).

Results showed that energy efficiency was unaffected, whether the energy was provide from carbohydrate-containing diets or carbohydrate-free, fatty diets; concluding that chicks utilised energy just as efficiently for fat synthesis as for growth. On the contrary, according to the findings of Carew *et al*. (1963) chicks utilised energy from fat more efficiently than from carbohydrate, when fat was incorporated at lower levels in the diet. The data also showed that chicks gained 0.40 kcal (0.002 MJ), on average, for each kilocalorie of ME consumed, irrespective of the level of protein or the source of non-protein energy. This is in agreement with Hill & Anderson (1958), where it was noted that chicks fed protein-deficient diets, deposited more calories as fat (Renner, 1964).

Supplemental fats have to be defined; as in most of the referred studies the primary effect noticed with age was the absorption of more saturated fats, such as tallow, with minimal effect on

utilisation of highly unsaturated fats such as maize oil or animal-vegetable blends. For example, poultry oil is highly unsaturated and its absorption should therefore, not be affected by age (Saleh *et al*., 2004).

CHAPTER 3

Trial 1: The effect of varying dietary energy levels on the performance of Hubbard Flex broilers

3.1 Abstract

Two experiments of identical design were conducted in floor pens to evaluate the effect of metabolisable energy (ME) level on broiler performance. The study was conducted over a period of five weeks, where five thousand seven hundred and sixty Hubbard Flex chicks were housed in 60 pens. Ninety six (96) chicks were placed in a randomly allocated pen at a stocking density of 16 birds/m^2. Each treatment was repeated once within a block, totalling to 5 replications/treatment/house. Broilers were fed *ad libitum* for a period of 35 days. Two isoprotein basal feeds were formulated and manufactured, one containing a high energy (HE) level, the other a low energy (LE) level. These basal diets were further diluted into 4 diets containing various percentages of energy (80% LE: 20% HE, 60% LE: 40% HE, 40% LE: 60% HE and 20% LE: 80% HE)). The crude protein and amino acid balance was kept constant across all treatments. There was no effect on body weight, body weight gain, feed intake or cumulative feed conversion ratio as the dietary energy levels increased. Although a linear positive response in broiler performance was noticed by Day 35 as dietary ME increased, the response was non-significant between treatments.

3.2 Introduction

As early as the 1950's, studies were conducted to determine the nutrient requirements of dietary ME levels, with concentrations ranging from: 12.5 MJ ME/kg of feed (Boekholt *et al*., 1994); 9.6 to 14.1 MJ ME/kg (Farrel *et al.*, 1973); 11.9 MJ ME/kg (ARC, 1963); 11.5 MJ ME/kg (NRC, 1963); 13.4 MJ ME/kg (NRC, 1971; Mendonca & Jensen, 1989). Energy recommendations are vital since energy alone contributes approximately 70% of total production cost in poultry diets. Accurate inclusion of energy levels contributes to optimum economical and production traits i.e. growth, feed efficiency and carcass quality (Saleh *et al*., 2004).

However, due to the differences of raw materials and their respective matrix values, dietary energy levels cannot be fixed as a constant value within a dietary formula. It is therefore advisable

to allow minimum and maximum limits for major nutrient recommendations to tolerate flexibility in diet formulation (Farrel *et al.*, 1973); as well as to accommodate varying dietary responses among different breeds, strains and sexes (Farrell, 1973).

Initial findings by Hill & Dansky (1954) indicated that chickens eat in order to meet their energy requirements, as long as all essential nutrients are provided. Therefore, at low energy (LE) concentrations broilers might not meet their dietary energy requirements if feed intake (FI) is similar compared to birds feeding on high energy (HE) diets. As a result, improved broiler performance would be expected for birds feeding on HE levels. According to Hill and Dansky (1954) birds feeding on LE diets would typically compensate by increasing their FI to meet their dietary energy requirements. These findings were supported by the NCR (1994) and Leeson *et al.* (1996). Despite these earlier findings, Saleh *et al*. (2004) stated that modern broilers are primarily selected to consume feed at high capacities, regardless of dietary energy levels; thereby attributing to improved performance without considering varying dietary energy levels. Later the NRC (1994) as well as Plumstead *et al*. (2007) reported a lack of an adjustment to FI caused by differing dietary metabolisable energy (ME) levels.

In spite of the improved performances noted by Saleh *et al*. (2004), Farrel *et al*. (1973) reported that an overconsumption of energy, possibly more than required for maximum growth rate, would be excessive and be deposited as carcass fat. Even with this finding, the efficiency of utilisation of ME is known to a lesser extent. The expectation is that nutrients are deposited within the growing and developing body tissue at a high efficiency, as the result of the selection for fast-growing broiler strains (Leeson & Summers, 2001; Lopez & Leeson, 2005).

The aim of this study was to optimise the energy density of isoprotein commercial rations fed to Hubbard Flex broilers in order to maximise performance. Consequently, only the dietary energy level (EL) varied among treatments fed to the broilers.

3.3 Materials and methods

3.3.1 Birds

Five thousand seven hundred and sixty Hubbard Flex were hatched at Midway Hatcheries, Limpopo. The birds were placed in 60 pens at day 0 and were housed at AFGRI Poultry's Trial facilities in Sundra, where they remained for the duration of the trial.

3.3.2 Husbandry

Birds were placed, managed and cared according to the standard operating procedures (SOP) of AFGRI Animal Feeds. The experimental procedures, [for placement, feeding, counting, recording of mortality, weighing of feed allocations and returns and weighing of birds, are as outlined in the Standard Operating Procedures: AFGRI Poultry Trial Facility No. 601-0 (Placement of Broiler Chicks); AFGRI Poultry Trial Facility No. 602-0 (Trial Routine Procedures) and AFGRI Poultry Trial Facility No. 604-0 (Trial Weighing Days)], were specifically established by AFGRI Poultry for their Trial facilities.

Each open-sided house was divided into 30 pens (3m x 2m) and the total number of 60 pens were equally divided into 5 blocks, totalling to 6 pens/ block. Ninety six (96) chicks were placed in a randomly allocated pen on Day 0 at a stocking density of 16 birds/m^2. Each treatment was repeated once within a block, totalling to 5 pens/treatment/house; thus 10 replicate pens/treatment.

Heat was supplied by means of a single Heat-Co boiler with plastic socks throughout the entire house. Temperature and ventilation were managed according to brooding practices starting at 35.5°C and reduced to 23°C, according to SOP No. 602-0 (Appendixes: Table 3-18). The lighting program (Appendixes: Table 3-19) started at 1 hour darkness at Day 0 increasing to a maximum of 4 hours in the final period. Feed and water was provided *ad libitum*.

3.3.3 Diet formulation and treatments

Dietary treatments consisted out of two basal feeds that were manufactured and expanded at a minimum of 95°C and subsequently blended into 4 additional diets to provide a total of 6 diets

Table 3-1 Raw material (ingredient) inclusion (%), calculated and analysed nutrient levels for the pre-starter and starter diets

Ingredients	**Pre-Starter (LE) Ingredient (%)**		**Pre-Starter (HE) Ingredient (%)**		**Starter (LE) Ingredient (%)**		**Starter (HE) Ingredient (%)**	
Maize	49.48		49.48		56.95		55.18	
Full fat soya (35.5%)	12.00		12.00		12.00		12.00	
Soybean oilcake (46%)	20.60		20.60		17.25		16.70	
Fish meal (65%)	5.43		5.43		6.03		6.68	
Sunflower oilcake (38%)	3.10		3.10		1.00		1.00	
White Gluten (60%)	4.60		4.60		2.25		2.18	
L threonine	0.041		0.041		0.032		0.032	
DL methionine	0.263		0.263		0.240		0.239	
Lysine HCl	0.274		0.274		0.199		0.183	
Soya bean oil	-		2.20		-		1.84	
Sodium bicarbonate	0.301		0.301		0.252		0.256	
Monocalcium phosphate	1.76		1.76		1.73		1.68	
Limestone	1.68		1.68		1.63		1.60	
Vitamin & minerals	0.187		0.187		0.187		0.187	
Nutrient Levels	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**
Dry matter	89.60	88.77	89.56	*90.67*	89.38	90.83	89.57	90.72
Crude protein	25.69	25.91	25.69	24.73	22.72	23.24	22.69	23.34
AME for chicks[1]	11.40		12.20		11.75		12.20	
Gross energy		16.63		17.05		16.28		16.86
Crude fibre	3.05	4.75	3.05	5.05	2.55	4.33	2.49	5.66
Fat (Ether extract)	5.08	5.43	7.23	7.13	5.16	5.37	6.96	7.60
Lysine[2]	1.35		1.35		1.20		1.20	
Methionine	0.67		0.67		0.60		0.61	
Total sulphur amino acids	0.99		0.99		0.88		0.88	
Threonine	0.84		0.84		0.74		0.74	
Tryptophan	0.24		0.24		0.21		0.21	
Arginine	1.43		1.43		1.27		1.27	
Isoleucine	0.94		0.94		0.82		0.82	
Valine	1.05		1.05		0.91		0.91	
Leucine	2.06		2.05		1.77		1.76	
Glycine and Serine	1.97		1.97		1.75		1.76	
C 18:2	2.21		2.88		2.24		2.76	
Calcium[3]	1.13	1.25	1.13	1.12	1.10	1.20	1.10	1.23
Potassium	1.00		1.00		0.90		0.89	
Chloride	0.20	0.227	0.20	0.217	0.20	0.211	0.20	0.202
Total Phosphorous	0.87	0.86	0.87	0.82	0.83	0.83	0.83	0.79
Retainable Phosphorous[4]	0.47		0.47		0.46		0.46	
Sodium	0.22	0.22	0.22	0.22	0.20	0.20	0.20	0.17

[1] OE for broiler chicks (CVB) [2] Amino acids availability for broiler chicks (CVB) for all amino acids [3] 0.08% Ca made available by addition of phytase enzyme[4] 0.08% P made available by addition of phytase enzyme [5] Additives: Pre-starter 0.09kg contribution; Starter 0.19kg contribution

Table 3-2 Raw material (ingredient) inclusion (%), calculated and analysed nutrient levels for the grower and finisher diets

Ingredients	**Grower (LE) Ingredient (%)**		**Grower (HE) Ingredient (%)**		**Finisher (LE) Ingredient (%)**		**Finisher (HE) Ingredient (%)**	
Maize	61.13		61.13		62.93		62.93	
Full fat soya (35.5%)	12.00		12.00		12.00		12.00	
Soybean oilcake (46%)	17.70		17.70		16.35		16.35	
Sunflower oilcake (38%)	1.00		1.00		1.00		1.00	
White Gluten (60%)	3.75		3.75		3.00		3.00	
L threonine	0.038		0.038		0.039		0.039	
DL methionine	0.212		0.212		0.201		0.201	
Lysine HCl	0.316		0.316		0.303		0.303	
Soya bean oil	-		2.23		0.78		3.08	
Sodium bicarbonate	0.146		0.146		0.123		0.123	
Monocalcium phosphate	1.67		1.67		1.41		1.41	
Limestone	1.45		1.45		1.30		1.30	
Vitamin & minerals	0.177		0.177		0.167		0.167	
Nutrient Levels	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**
Dry matter	89.34	89.86	89.28	90.95	89.31	89.37	89.27	89.43
Crude protein	20.32	19.71	20.32	19.44	19.32	19.60	19.32	19.34
AME for chicks[1]	11.85		12.65		12.15		12.95	
Gross energy		16.24		16.59		16.33		16.88
Crude fibre	2.68	3.67	2.64	3.87	2.66	3.50	2.66	3.57
Fat (Ether extract)	4.79	4.80	7.07	6.83	5.55	5.80	7.70	7.45
Lysine[2]	1.05		1.05		1.00		1.00	
Methionine	0.50		0.50		0.48		0.48	
Total sulphur amino acids	0.77		0.77		0.73		0.73	
Threonine	0.65		0.65		0.62		0.62	
Tryptophan	0.18		0.18		0.17		0.17	
Arginine	1.11		1.11		1.06		1.06	
Isoleucine	0.73		0.73		0.69		0.69	
Valine	0.81		0.80		0.76		0.76	
Leucine	1.70		1.70		1.60		1.60	
Glycine and Serine	1.50		1.50		1.43		1.43	
C 18:2	2.35		3.02		2.59		3.25	
Calcium[3]	0.87	0.88	0.87	0.96	0.77	0.87	0.77	0.80
Potassium	0.88		0.88		0.85		0.85	
Chloride	0.20	0.25	0.20	0.248	0.20	0.276	0.20	0.233
Total Phosphorous	0.71	0.69	0.71	0.67	0.65	0.64	0.65	0.63
Retainable Phosphorous[4]	0.36		0.36		0.32		0.32	
Sodium	0.17	0.13	0.17	0.13	0.16	0.15	0.16	0.15

[1] OE for broiler chicks (CVB) [2] Amino acids availability for broiler chicks (CVB) for all amino acids [3] 0.08% Ca made available by addition of phytase enzyme[4] 0.08% P made available by addition of phytase enzyme [5] Additives: Grower 0.09kg contribution; Finisher 0.09kg contribution

with differing dietary energy levels. Treatment A provided the lowest level of dietary energy – Low Energy (LE) and Treatment F, the highest level – High Energy (HE), at each of the four feeding phases (Table 3-1 and Table 3-2).

Prior to diet formulation, the total and digestible AA composition of all feed ingredients were calculated as a percentage of the analysed CP content and calculated total AA content, respectively, using standard procedures (CVB, 2001). In order to better assess the specific effects for ME and to minimise extraneous variation, the inclusion of primary ingredients such as maize and soy bean meal differed marginally, if at all. The increase in the proportion of dietary ME between Treatment A and F was derived from fat, more specifically from soy oil that was added to Treatment F only. Similarly, to prevent treatment difference arising from moisture difference between the contents of the basal diets, a set percentage of moisture by the addition of water, was added to each basal diet. Additionally, to further minimise variation among dietary treatments that could have originated as a result from the batching and mixing of ingredients, a dilution technique was applied.

This technique involved the blending Treatment A and F to create 4 intermediate treatments (Diet B, C, D and E). Treatment B was a blend of (80% LE: 20% HE); Treatment C was a blend of (60% LE: 40% HE); Treatment D was a blend of (40 % LE: 60% HE) and Treatment E was a blend of (20% LE: 80 % HE).

All feed was mixed at AFGRI Animal Feeds (Isando, South Africa). Phases were fed as follows: Pre-starter (0 to 10 days), Starter (11 to 18 days), Grower (19 to 28 days) and Finisher (29 to 35 days) periods.

a. Feed Analyses

Table 3-3 Calculated apparent metabolisable energy (AME; MJ/kg feed) of the different treatments and phases

	AME (MJ/kg)			
	Pre-starter	Starter	Grower	Finisher
Treatment				
A (100% Low Energy; LE)	11.40 MJ	11.70 MJ	11.85 MJ	12.15 MJ
B (80% LE:20% HE)	11.56 MJ	11.80 MJ	12.01 MJ	12.31 MJ
C (60% LE:40% HE)	11.72 MJ	11.90 MJ	12.17 MJ	12.47 MJ
D (40% LE:60% HE)	11.88 MJ	12.00 MJ	12.33 MJ	12.63 MJ
E (20% LE:80% HE)	12.04 MJ	12.10 MJ	12.49 MJ	12.79 MJ
F (100% High Energy; HE)	12.20 MJ	12.20 MJ	12.65 MJ	12.95 MJ

Representative samples of the different feeds were collected during the respective feeding phases, before the birds had access to the feed. Each sample was ground and analysed for dry matter (DM), ash, crude protein (CP), gross energy (GE), crude fibre (CF), ether extract (EE), calcium (Ca), phosphorus (P), sodium (Na) and chloride (Cl). The analysis was done at NutriLab (Department of Animal and Wildlife Sciences, University of Pretoria). Moisture was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 934.01). Dry matter and ashing were analysed according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 942.05). Crude protein was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 988.05). Gross energy was determined using the MC – 1000 Modular Calorimeter. Crude fibre was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 962.09). The Dosi fibre system was used to determine the crude fibre percentage. Crude fat was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 920.39).

Samples were prepared for calcium and sodium analysis following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 935.13). Samples were prepared for phosphorus analysis following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 968.08.D.b.). Calcium was determined according to the Giron, H.C's official method of analysis (Giron. H.C., 1973, Perkin Elmer Atomic Spectrophotometer). Phosphorus was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 965.17). Sodium was determined according to the Giron, H.C's official method of analysis (Giron. H.C., 1973, Perkin Elmer Atomic Spectrophotometer). Chloride was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 969.10).

3.3.4 Performance measurements

All live birds were weighed weekly on a per pen basis on day 7, 14, 21, 28 and 35 to calculate mean weekly body weights (BW) and weekly body weight gain. Remaining feed left in feeders of each pen were weighed back weekly as well as at the change of each feed phase (day 10, end of pre-starter; day 18, end of starter and day 28, end of the grower). Weekly feed intake (WFI), cumulative feed intake (CFI), weekly feed conversion ratio (FCR) and cumulative feed conversion ratio (CFCR) were calculated from the amount of feed provided and weighted back. Mortalities

were collected twice daily, weighed and recorded; mortalities that were noted on a weighing day were recorded as a mortality on that day.

3.4 Statistical analyses

To assess the effect of energy on performance traits, a randomised block design was used to evaluate the data. The trial data was analysed as a mixed model using the Fit Model procedure with the REstricted Maximum Likelihood (REML) method. The fixed factors per house were Treatment (levels = 6) and House (levels = 2); and Block was included as a random factor (levels = 5). Although House could also be considered a random factor, it had to be included in the model as a fixed factor as there were only two levels.

The treatments in this trial were structured and therefore simple analysis of treatment means was the most appropriate statistical analysis to be used on the data. Data were subjected to a statistical model and an analysis of variance (ANOVA) was done on all treatment means; where significant differences were indicated, post hoc multiple-comparison tests such as student t-test or Tukey's test were applied. The student t-test was an appropriate multi-comparison test for finding significant differences within a factor of 2 levels. The Tukey's test was an appropriate test to use when a factor has 3 or more levels within. The confidence level was set at 95%.

3.5 Results

In general, analysed values for DM, CP, GE, CF, EE, Ca, Cl, P and Na were close to the formulated values of Treatment A and F (Table 3-1; Table 3-2; Appendix: Table 3-10; Appendix: Table 3-11; Appendix: Table 3-12; Appendix: Table 3-13). Treatment F from all the feeding rations analysed higher for GE and EE compared to Treatment A.

Although differences in both BW and body weight gain approached significance from Treatment A to F, no significant differences were recorded throughout the trial ($P > 0.05$, Table 3-4; Table 3-5). A numerical difference was recorded weekly for BW between Treatment F and A; Treatment F birds finally weighing heavier on day 35 (1934.0 g) compared to Treatment A (1898.10 g), but with no significant difference between the results ($P > 0.05$). Similarly, Treatment F birds gained 77.0 g/bird/d by day 35, compared to the 72.6 g/bird/d of Treatment A birds, also at a non-significant value ($P > 0.05$). Despite this NS difference in BW and average daily gain between

Treatments at day 35, Figure 3-1 illustrates the increase in BW (R^2 = 0.78) as FI intake, and subsequent EI, increases (R^2 = 0.71).

Table 3-4 Mean weekly body weight (g) of mixed sex Hubbard Flex broilers fed increasing levels of dietary energy

	Body weight (g)					
	0 d	7 d	14 d	21 d	28 d	35 d
Treatment						
A (100% Low Energy; LE)	40.8	162.1	432.8	846.5	1388.9	1898.1
B (80% LE:20% HE)	41.0	161.8	432.6	852.5	1387.1	1922.1
C (60% LE:40% HE)	41.0	161.6	431.3	848.1	1383.7	1911.7
D (40% LE:60% HE)	41.1	164.5	435.7	856.0	1400.2	1923.3
E (20% LE:80% HE)	40.7	163.7	437.8	862.9	1405.1	1926.1
F (100% High Energy; HE)	40.9	164.3	438.0	857.9	1400.7	1934.0
SEM	0.5	3.3	9.4	17.5	24.7	35.5
F-Prob:						
Treatment	0.521	0.168	0.506	0.314	0.330	0.153
Block	0.991	0.236	0.302	0.511	0.712	0.559
R^2 (% Var)	0.47	0.60	0.40	0.34	0.32	0.37

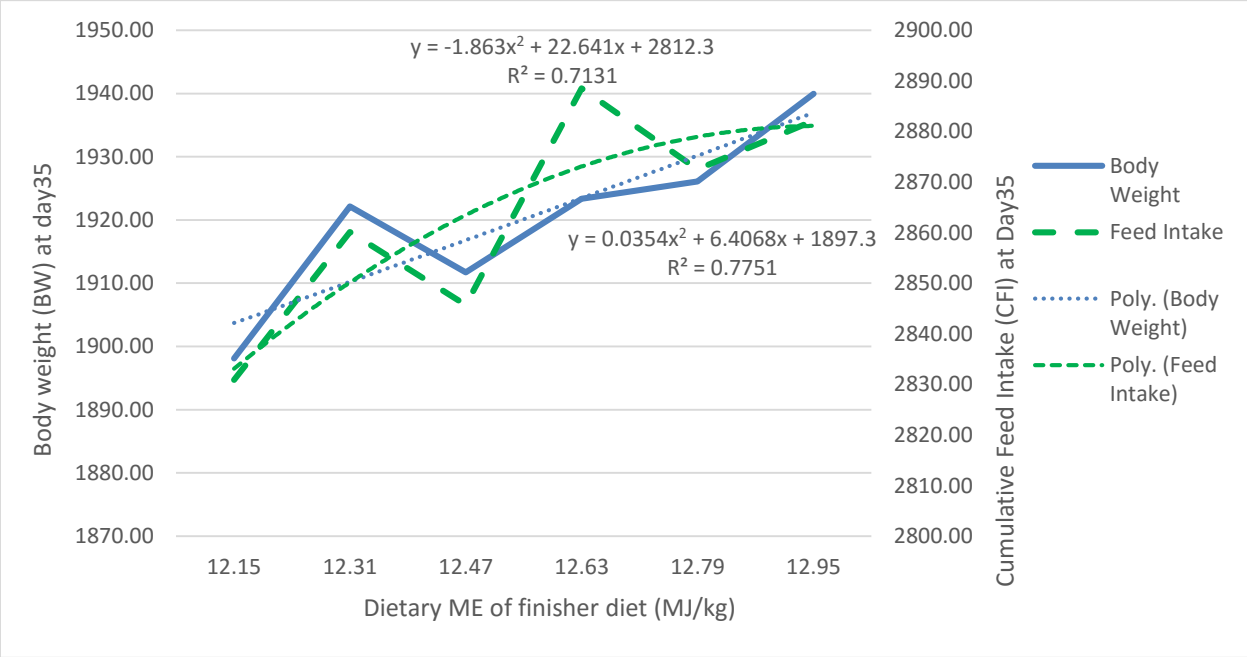

Figure 3-1 Body weight and cumulative feed intake (- - -) (vertical bars) of mixed sex broiler chickens at day 35 of the experiment, as affected by increasing ME density from 12.15 to 12.95 MJ/kg. Polynomial trend line for BW – R^2 = 0.78; CFI – R^2 = 0.71

Cumulative feed intake (CFI) (g/bird) showed no significant differences between treatments for the entire duration of the trial (P > 0.05, Table 3-6), although Treatment F birds consumed 51.79

g more feed by day 35 compared to Treatment A. Total FI of Treatment F birds was 2882.11 g/bird over 35 days compared to the 2830.90 g/bird for Treatment A birds (P > 0.05).

Table 3-5 Mean weekly body weight gain (g/bird/d) of mixed sex Hubbard Flex broilers fed increasing levels of dietary energy

	Body weight gain (g/bird/d)				
	0 - 7 d	7 - 14 d	14 - 21 d	21 - 28 d	28 - 35 d
Treatment					
A (100% Low Energy; LE)	15.1	38.6	59.1	77.4	72.6
B (80% LE:20% HE)	15.1	38.7	60.0	76.4	76.5
C (60% LE:40% HE)	15.1	38.5	59.5	76.6	75.5
D (40% LE:60% HE)	15.5	38.8	60.0	77.9	75.0
E (20% LE:80% HE)	15.3	39.1	60.8	77.3	74.2
F (100% High Energy; HE)	15.4	39.1	60.0	77.5	77.0
SEM	0.4	1.0	1.4	1.9	3.4
F-Prob:					
Treatment	0.172	0.726	0.219	0.502	0,066
Block	0.224	0.210	0.451	0.223	0.478
R^2 (% Var)	0.62	0.31	0.33	0.45	0.50

Table 3-6 Mean cumulative feed intake (g/bird) of mixed sex Hubbard Flex broilers fed increasing levels of dietary energy

	Cumulative feed intake (g/bird)				
	0 - 7 d	0 - 14 d	0 - 21 d	0 - 28 d	0 - 35 d
Treatment					
A (100% Low Energy; LE)	136.3	464.6	1043.8	1886.5	2830.9
B (80% LE:20% HE)	139.0	463.9	1047.5	1890.1	2860.1
C (60% LE:40% HE)	138.3	467.3	1048.6	1889.7	2845.7
D (40% LE:60% HE)	137.0	470.7	1059.3	1917.7	2888.5
E (20% LE:80% HE)	138.6	471.2	1057.3	1907.5	2872.5
F (100% High Energy; HE)	140.0	472.0	1061.2	1914.9	2882.1
SEM	3.6	9.8	18.2	32.6	49.4
F-Prob:					
Treatment	0,249	0.286	0.192	0.129	0.099
Block	0,037	0.232	0.317	0.434	0.398
R^2 (% Var)	0,66	0.43	0.36	0.42	0.38

No significant difference was recorded for cumulative feed conversion ratio (CFCR) between treatments by Day 35 when Treatment A showed a CFCR of 1.53 points compared to the CFCR of 1.52 points for Treatment F (P > 0.05, Table 3-7).

Despite the measurable increase in EL from Treatment A to F, there was no significant effect on daily mortalities (P > 0.05, Table 3-8).

Table 3-7 Mean cumulative feed conversion ratio (g feed/g gain) of mixed sex Hubbard Flex broilers fed increasing levels of dietary energy

	Cumulative feed conversion ratio (CFCR) (g feed/g gain)				
	0 - 7 d	0 - 14 d	0 - 21 d	0 - 28 d	0 - 35 d
Treatment					
A (100% Low Energy; LE)	1.13[ab]	1.19	1.30	1.40	1.53
B (80% LE:20% HE)	1.15[b]	1.18	1.29	1.40	1.52
C (60% LE:40% HE)	1.15[ab]	1.20	1.30	1.41	1.52
D (40% LE:60% HE)	1.11[a]	1.19	1.30	1.41	1.53
E (20% LE:80% HE)	1.13[ab]	1.19	1.29	1.40	1.53
F (100% High Energy; HE)	1.13[ab]	1.19	1.30	1.41	1.52
SEM	0.03	0.02	0.02	0.02	0.02
F-Prob:					
Treatment	0.038	0.887	0.458	0.612	0.287
Block	0.016	0.053	0.335	0.424	0.425
R^2 (% Var)	0.52	0.46	0.38	0.31	0.64

[a,b] Column means with common superscripts do not differ significantly ($P < 0.05$).

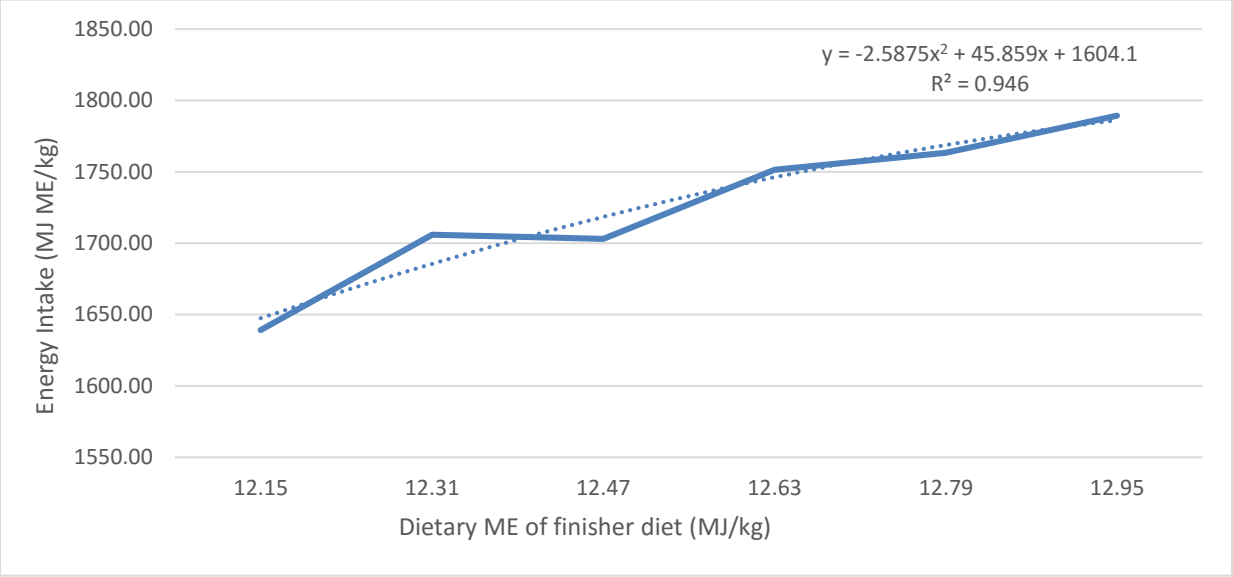

Figure 3-2 Weekly energy intake (WEI) (vertical bar) of mixed sex broiler chickens at day 35 of the experiment, as affected by increasing the ME density from 12.15 to 12.95 MJ/kg. Polynomial trend line for WEI – $R^2 = 0.95$

As for Weekly Energy Intake (WEI), a significant increase from Treatment A to F was recorded throughout the trial period. By day 35, Treatment A consumed 1639.23 kJ ME/bird/week compared to Treatment F's 1789.32 kJ ME/bird/week, with the majority of treatments in between

increasing from Treatment A to F ($P \leq 0.001$, Table 3-9). This is also illustrated in Figure 3-2, where the EI increases almost linearly ($R^2 = 0.95$) as the energy content increase from Treatment A to Treatment F in the finisher diet.

Table 3-8 Mean cumulative mortality (% birds placed) of mixed sex Hubbard Flex broilers fed increasing levels of dietary energy

	Cumulative mortality (% birds placed)				
	0 - 7 d	0 - 14 d	0 - 21 d	0 - 28 d	0 - 35 d
Treatment					
A (100% Low Energy; LE)	0.63	1.15	1.77	3.02	5.10
B (80% LE:20% HE)	0.31	0.73	1.25	3.54	6.77
C (60% LE:40% HE)	0.31	0.84	1.67	3.02	4.69
D (40% LE:60% HE)	0.21	0.73	1.67	3.23	5.21
E (20% LE:80% HE)	0.10	0.63	1.46	3.65	5.94
F (100% High Energy; HE)	0.21	0.94	1.87	3.23	5.41
SEM	0.61	0.84	1.33	1.90	2.97
F-Prob:					
Treatment	0.518	0.782	0.919	0.965	0.682
Block	0.111	0.187	0.146	0.114	0.452
R^2 (% Var)	0.37	0.40	0.31	0.33	0.27

Table 3-9 Mean weekly energy intakes (kJ ME/bird/week) of mixed sex Hubbard Flex broilers fed increasing levels of dietary energy

	Weekly energy intakes (kJ ME/bird/week)				
	0 - 7 d	7 - 14 d	14 - 21 d	21 - 28 d	28 - 35 d
Treatment					
A (100% Low Energy; LE)	194.2^{c}	544.5^{b}	974.8^{d}	1426.5^{d}	1639.2^{c}
B (80% LE:20% HE)	200.8^{b}	544.4^{b}	993.0^{cd}	1445.7^{cd}	1705.9^{b}
C (60% LE:40% HE)	202.6^{b}	556.7^{b}	1000.1^{c}	1462.2^{c}	1703.1^{b}
D (40% LE:60% HE)	203.5^{b}	570.4^{a}	1023.4^{b}	1512.2^{b}	1751.5^{a}
E (20% LE:80% HE)	208.7^{a}	574.1^{a}	1030.0^{ab}	1516.6^{ab}	1763.3^{a}
F (100% High Energy; HE)	213.4^{a}	579.9^{a}	1046.7^{a}	1542.7^{a}	1789.3^{a}
SEM	5.31	13.41	19.92	28.94	45.23
F-Prob:					
Treatment	0.000	0.000	0.000	0.000	0.000
Block	0.038	0.225	0.346	0.355	0.654
R^2 (% Var)	0.79	0.71	0.72	0.80	0.66

^{a,b,c,d} Column means with common superscripts do not differ significantly ($P < 0.05$).

3.6 Discussion

The analysed nutrient levels were relatively close to calculated values. There were limitations with regard to the feed analysis i.e. dietary energy was analysed by means of measuring the GE, even though dietary energy was formulated on an AME (MJ/kg) basis. Despite relying on GE to

indicate the differences in dietary EL, all Treatment F feeds analysed higher in energy than Treatment A.

The results of this experiment showed that BW, body weight gain and FI responded linearly by Day 35 as dietary energy levels increased between Treatments A to F. All differences were non-significant ($P > 0.05$).

Conventional early theories such as Hill and Dansky (1954), stated it was generally assumed for many years that chickens eat to meet their energy needs. The theory of Feed Intake Regulation by Emmans (1981) stated that feeding a low energy diet will lead to an increase in feed intake to acommodate for the deficient dietary energy as primary nutrient. This was supported by the NRC (1994) and Leeson *et al*. (1996), who published results where poultry adjusted their FI to differences in the dietary ME density in order to maintain a constant ME intake. However, FI is determined by a combination of dietary, environmental and physiological factors. Saleh *et al*. (2004) stated that more recent research showed an increased growth rate and improved feed conversion with increasing levels of dietary energy. This is the outcome of the modern broiler primarily selected for growth rate and meat production and consequently, do not regulate voluntary feed intake (VFI) adequately to achieve energy balance (Richards, 2003); but consume feed at almost full capacity regardless of the dietary energy. Despite this, the cumulative feed conversion ratio (CFCR) between broilers from Treatment A and F, in this study, did not differ significantly.

The NRC (1994) also stipulated an apparent lack of an effect of dietary ME on FI in the modern broiler. Differences in the effect of ME density on FI observed by other authors may have been caused by differences in the range of dietary ME assessed, the age of the broilers, as well as by variation in the dietary formulation techniques used in their respective experiments. Leeson *et al*. (1996) reported a 200 g lower FI by Day 25, when the dietary ME was increased from 11.15 MJ to 13.62 MJ/kg. This 2.47 MJ/kg increase was considerably more than the difference between 12.15 MJ/kg and 12.95 MJ/kg of the Finisher diets in the present study. It is, therefore, possible that the modern broiler still have the ability to adapt FI according to energy density of the feed over wide ranges of energy levels, but lacks the sensitivity to do so when changes in energy levels are small.

It has been shown that growing chicks can tolerate high levels of dietary fat if the nutrient balance in the feed is maintained. Even further, researchers observed the complete replacement of carbohydrates with fat in the diet of a chick, without affecting the rate of growth. The study data indicated the isocaloric substitution of carbohydrates for fat was successful on condition that the protein level of the diet was maintained at a minimum to prevent a decrease in growth. Therefore, the result between the "carbohydrate free" diet and the standard showed that the requirement for protein was no different; also that chicks fed the "carbohydrate free" diet utilised the protein just as efficiently as chicks fed the standard carbohydrate-containing diet. This concluded that the requirement of carbohydrate as an energy source could be met without diverting AA from protein to carbohydrate synthesis, when the protein is supplied by a mixture of protein sources such as soybean and synthetic AA. Since the energy constituent of any broiler feed contributes to approximately 70% of the production costs, alternative sources of dietary energy remains an incentive. Especially since the requirement for energy increases as the bird's age, the replacement of carbohydrates with fat early on is a possible cost saving initiative (Saleh *et al.*, 2004).

Soybean oil was added to Treatment F in all feeding phases to achieve the required dietary ME. The maximum level of oil included was 3.12% for the Finisher diet. Leeson *et al*. (1996) achieved changes in the dietary ME by increasing the dietary inclusion of an animal-vegetable fat blend from 1.15% to 8.65%. This inclusion caused a linear correlation between dietary ME and added fat in their study; making it difficult to separate the effect of dietary ME and added fat on FI.

In the present study, the soya oil was added post-pelleting and was low enough not to have a negative effect on pellet quality of Treatment F diets; as opposed to the findings by Skinner *et al*. (1992), who noted a dramatic decline in the physical quality of the feed pellets when oil levels greater than 5%, were added to the diet. Additionally, researchers demonstrated the depression in feed intake due to a reduction in pellet quality in feeds with higher dietary energy levels; possibly caused by the excessive oil levels in these diets (Saleh *et al*. 2004).

Conversely, the added oil in the Treatment F diets in this experiment, appears to be one of the drivers for the increased intake. The experimental diets utilised in Plumstead *et al.* (2007) was formulated in such a manner that the proportion of dietary ME derived from added fat was fixed. It was concluded that the similar FI observed across the experimental diets ranging from 12.38

MJ/kg to 13.21 MJ/kg, proved that the broilers did not adjust their FI by Day 21 in response to increasing dietary ME concentration.

Previous research illustrated an upper limit to the amount of energy a broiler would be able to consume before performance is affected (Farrell, 1973; Farrell, 1974; Saleh *et al*., 2004). Although energy levels in this study were not sufficient to cause a reduction in performance, it is possible that the higher dietary energy levels caused an increase in carcass fat. ME intake is well documented to influence body composition and performance of growing broilers, but less information is available on utilisation of ME intake (Lopez & Leeson, 2005).

Growing broilers deposit nutrients into body tissues, such as fat and protein, quite efficiently relative to other poultry species. ME intake is generally partitioned into energy retained (ER) in body tissues, mainly as fat (ERF) and protein (ERP), and as heat production. Energy supply has a direct effect on the relation between EFR and ERP (Boekholt *et al.* 1994; Lopez & Leeson, 2005). ERF and energy supply proves to be linear at all values of energy intake beyond a specific dietary energy value, i.e. increasing the energy supply results in a supplementary retention of mainly fat. Below this dietary energy value, ERF = 0, and therefore only protein is retained at the expense of mobilised fat (Boekholt *et al.,* 1994). Even so, it is generally accepted that carcass fatness will not change as long as the energy to protein ratio (E: P) does not change. If the ratio is not constant, carcass fatness increases as the dietary energy level increases and the ratio widens (Teeter *et al*., 1996; Saleh *et al*., 2004).

One of the frequently expressed concerns regarding the use of high dietary energy levels is the potential impact on mortality and the incidence of leg disorders with the increase of dietary ME. There were no significant differences among the treatments on mortality; neither a noteworthy problem with leg disorders, although no actual leg scoring was done on the birds.

As a consequence of the lack of response in FI from Treatment A to F weekly energy intake (WEI) increased significantly at all weekly recordings ($P < 0.001$), as dietary EL increased.

3.7 Conclusion

It can be concluded that despite early theories about the regulation of FI, the continuous selection for rapid growth in juvenile broilers may have altered a broiler's ability to regulate FI according to dietary ME densities.

Even though dietary ME levels increased from 12.15 MJ/kg to 12.95 MJ/kg by the addition of extra vegetable oil to the diets, feed intake was not significantly affected. As a result, ME intake of broilers receiving the HE diets, was significantly higher than those on LE diets. This, did not translate in improved broiler performance, although a non-significant linear response was noted. In practice, the nutrient density of diets will be limited by the associated exponential increase in operational cost as dietary concentration of ME increases. Since broiler performance was shown to be significantly non-responsive to the increased dietary ME, it would not be beneficial to feed a high energy diet such as 12.95 MJ/kg used in this study.

CHAPTER 4

Trial 2: The effect of varying dietary protein levels on the performance of Hubbard Flex broilers

4.1 Abstract

Two experiments of identical design were conducted in floor pens to evaluate the effect of crude protein (CP) level on broiler performance. The study was conducted over a period of five weeks, where five thousand seven hundred and sixty Hubbard Flex chicks were housed in 60 pens. Ninety six (96) chicks were placed in a randomly allocated pen at a stocking density of 16 birds/m^2. Each treatment was repeated once within a block, totalling to 5 replications/treatment/house. Broilers were fed *ad libitum* for a period of 35 days. Two isocaloric basal feeds were formulated and manufactured, one containing a high protein (HP) level, the other a low protein (LP) level. These basal diets were further diluted into 4 diets containing various percentages of protein (80% LP: 20% HP, 60% LP: 40% HP, 40% LP: 60% HP and 20% LP: 80% HP)).The dietary metabolisable energy (ME) was kept constant across all treatments. Birds from Treatment F had the highest BW at Day 21 with 900.97 g compared to Treatment A birds on 858.85 g. By Day 35 the Cumulative Feed Conversion Ratio (CFCR) for Treatment F was 1.50; 9 points better than Treatment A. There was no significant effect of treatments on the mortality of birds.

4.2 Introduction

Broiler performance in terms of economic interest, such as body weight gain and feed efficiency, can be optimised by increasing the dietary amino acid (AA) concentrations, improving the AA balance, or both. Modern high-yielding broilers have shown increased responsiveness to AA density, especially for lysine (Lys) (Vieira & Angel, 2012). The increased dietary Lys and other AA's at early stages of the growing point have showed positive carry-over effects on performance during later periods (Vieira & Angel, 2012). The use of crude protein (CP) level as the indication of dietary protein in feed formulation causes for the assumption that protein is simply the sum of essential amino acids (EAA) and non-essential amino acids (NEAA) and are either naturally present or added; nonetheless, both are required for maximum growth and performance (Pym *et al*., 1984). Literature recognises that individual EAA are a function of the total CP level, i.e. amino acid (AA) requirements are proportional to the CP content of the diet (NRC, 1994). This biological

function is needed to synthesise body proteins and has been referred to as the “ideal protein (AA) balance” (Pym *et al*., 1984). Multiple factors such as the diet, environment and genetics influence AA requirements; thereby complicating the determination of exact amounts of any AA required, especially when relying on the dose-response experiments (Mack *et al*., 1999).

Additionally, variables such as the period of growth (age), gender and criterion response can affect the protein balance as well. For example, males are known to have higher protein requirements than females and maximal feed efficiency (i.e., gain: feed ratio) requires a higher dietary lysine level than maximal body weight gain (Boekholt *et al*., 1994). It is therefore possible to list the order of protein requirement from high to low with respect to the response to differing CP levels in the diet (Pesti, 2009).

1. For body weight (BW)
2. To maximise feed utilisation
3. Maximise lean carcass weight and
4. Minimise carcass fat (Pesti, 2009).

Inversely, the required level of lysine to maximise specific performance traits a greater requirement to minimise abdominal fat percentage and less to maximise breast meat yield and body weight gain (Vieira & Angel, 2012).

Pig nutritionists were the first to clarify the concept that the AA requirement can be expressed as a ratio to lysine for different weight categories (Mendonca & Jensen, 1989). Founded on the perception that even though the AA requirements change because of various factors, the ideal EAA: lysine ratio will only be affected marginally within a certain age period (Mack *et al*., 1999). Early studies in 1960’s and 1970’s hypothesised that, when protein synthesis becomes limited by the first-limiting EAA, AA present in excess of the resulting requirement enter the AA pool of substrates. From this pool, AA then become available as resources such as energy producing systems. Alternatively, excessive AA would result in a greater heat increment compared to a balanced AA diet, since energy would be used to eliminate the excessive AA. Conversely, surplus AA would enter a relative process of catabolism, thus sparing the utilisation of other substrates for heat production (Macleod, 1997).

AA utilisation is most efficient when all AA are provided exactly or slightly below, the recommendations for protein accretion and maintenance. Additionally, formulating diets that do not exceed AA requirements, also results in lower nitrogen excretion. This method of controlling NH_3 production is possibly the most economical and efficient according to Ferguson *et al.* (1998). Excessive nitrogen (N) losses are due to an imbalance between EAA and NEAA, originating from the oversupply of the required AA. In effect, the closer the available EAA in the diet match the required amount for growth or maintenance, the less AA will be degraded and excreted as NH_3. These diets are also more economically feasible (Corzo *et al*., 2004). Current practice, is to supply crude protein slightly in excess of requirements, because formulated rations must satisfy practical least cost rather than maximum protein accretion levels. Even so, balancing the AA can either be achieved by formulating broiler diets on a digestible AA basis, reducing the amount of dietary CP, reducing the CP in the feeding phases as birds age or the use of synthetic AA (i.e. methionine, lysine and threonine) (Ferguson *et al*., 1998).

Early research by Fraps (1943) stipulated the relationship between dietary protein and broiler performance, showing that the manipulation of dietary protein adversely affects performance. Noticeable differences in BW, feed intake (FI) and carcass composition with particular reference to abdominal fat were observed and this developed as a pattern over time to modern poultry science. In more recent studies, Baker (2008) stated that protein gain (as a percentage of body weight gain) decreases as chicks grow and BW increases, thus the requirements for all AA decrease with age and BW.

According to a broiler study by Ferguson *et al*. (1998) at day 42 of age there were significant differences in most of the production traits, between High, Medium and Low density treatments, confirming the idea that there is a critical dietary CP level below which a bird's performance will decline. In another study dietary CP below 18.8% from 3 to 6 weeks of age, resulted in adverse effects on the BW, daily gain, FI and feed conversion ratio (FCR). The opposite is possible where improved growth and meat yield were the result of diets higher in nutrient density compared to the NRC (1994) (Dozier *et al*., 2006). Although the NRC (1994) was compiled over 20 years ago, they still approved the practice of decreasing protein density within the three growth periods, i.e. starter phase (0 – 3 weeks), grower phase (3 – 6 weeks) and finisher phase (6 – 8 weeks).

Dietary CP and AA are known to have a more indirect effect of FI compared to dietary energy. AA imbalances have the ability to alter FI rapidly. This is even possible in small chicks, suggesting

that a subsequent outcome, positive (improvement in growth) or negative (growth depression), is not the direct response to the imbalance itself (Ferket & Gernat, 2006).

The aim of this study was to examine the effects of high protein (HP) and low protein (LP) level at fixed energy levels on broiler feed intake and performance.

4.3 Materials and methods

4.3.1 Birds

Five thousand seven hundred and sixty Hubbard Flex chicks were hatched at Midway Hatcheries, Limpopo. The birds were placed in 60 pens at day 0 and were housed at AFGRI Poultry's Trial facilities in Sundra, where they remained for the duration of the trial.

4.3.2 Husbandry

Birds were placed, managed and cared for according to the standard operating procedures (SOP) for AFGRI Animal Feeds. The experimental procedures, for placement, feeding, counting, recording of mortality, weighing of feed allocations and returns and weighing of birds, are as outlined in the Standard Operating Procedures: AFGRI Poultry Trial Facility No. 601-0 (Placement of Broiler Chicks); AFGRI Poultry Trial Facility No. 602-0 (Trial Routine Procedures) and AFGRI Poultry Trial Facility No. 604-0 (Trial Weighing Days), were specifically established by AFGRI Poultry for their Trial facilities.

Each open-sided house was divided into 30 pens (3m x 2m) and the total number of 60 pens were equally divided into 5 blocks, totalling to 6 pens/ block. Ninety six (96) chicks were placed in a randomly allocated pen on Day 0 at a stocking density of 16 birds/m^2. Each treatment was repeated once within a block, totalling to 5 pens/treatment/house; thus 10 replicate pens/treatment.

Heat was supplied by means of a single Heat-Co boiler with plastic socks throughout the entire house. Temperature and ventilation were managed according to brooding practices starting at 35.5°C and reduced to 23°C, according to SOP No. 602-0 (Appendixes: Table 3-18). The lighting

program (Appendixes: Table 3-19) started at 1 hour darkness at Day 0 increasing to a maximum of 4 hours in the final period. Feed and water was provided *ad libitum*.

4.3.3 Diet formulation and treatments

Dietary treatments consisted of two basal feeds that were manufactured and expanded at a minimum of 95°C and subsequently blended into 4 additional diets to provide a total of 6 diets with differing dietary CP and AA levels. Treatment A provided the lowest level of dietary CP – Low Protein (LP) and Treatment F, the highest level – High Protein (HP), at each of the four feeding phases (Table 4-1 and Table 4-2).

Prior to diet formulation, the total and digestible AA composition of all feed ingredients were calculated as a percentage of the analysed CP content and calculated total AA content, respectively, using standard procedures (CVB, 2001). In order to better assess the specific effects for CP and the AA balance as well as minimising extraneous variation, the use of primary ingredients such as maize and soy bean meal were similar in basal diets. The increase in the proportion of dietary CP and AA between Treatment A and F, was derived from multiple ingredients as well as synthetic AAs. Similarly, to prevent treatment difference arising from moisture difference between the contents of the basal diets, a set percentage of moisture by the addition of water, was added to each basal diet. Additionally, to further minimise the variation between dietary treatments that could have originated as a result from the batching and mixing of ingredients, a dilution technique was applied.

This technique involved the blending Treatment A and F to create 4 intermediate treatments (Diet B, C, D and E). Treatment B was a blend of (80% LP: 20% HP); Treatment C was a blend of (60% LP: 40% HP); Treatment D was a blend of (40% LP: 60% HP) and Treatment E was a blend of (20% LP: 80% HP).

All feed was mixed at AFGRI Animal Feeds (Isando, South Africa). Phases were fed as follows: Pre-starter (0 to 10 days), Starter (11 to 18 days), Grower (19 to 28 days) and Finisher (29 to 35 days) periods.

Table 4-1 Raw material (ingredient) inclusion (%), calculated and analysed nutrient levels for the pre-starter and starter diets

Ingredients	**Pre-Starter (LP) Ingredient (%)**		**Pre-Starter (HP) Ingredient (%)**		**Starter (LP) Ingredient (%)**		**Starter (HP) Ingredient (%)**	
Maize	54.83		50.00		57.30		51.38	
Full fat soya (35.5%)	7.00		8.75		10.00		12.00	
Soybean oilcake (46%)	19.80		21.80		20.20		22.20	
Fish meal (65%)	5.50		6.50		1.00		2.00	
Sunflower oilcake (38%)	4.00		4.00		1.00		1.00	
Gluten (60%)	1.45		1.98		3.00		4.00	
Full fat maize germ	1.00		-		1.85		1.03	
L threonine	0.069		0.073		-		0.058	
DL methionine	0.205		0.232		0.126		0.234	
Lysine HCl	0.277		0.275		0.175		0.323	
Soya bean oil	-		0.50		-		-	
Salt	0.098		0.075		0.228		0.203	
Sodium bicarbonate	0.305		0.308		0.225		0.228	
Monocalcium phosphate	1.70		1.55		1.93		1.78	
Limestone	1.53		1.48		1.63		1.58	
Vitamin & minerals	0.187		0.187		0.187		0.187	
Nutrient Levels	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**
Dry matter	89.40	89.82	89.57	90.30	89.40	90.72	89.62	90.74
Crude protein	22.35	23.40	24.48	25.24	20.66	21.72	23.39	24.16
AME for chicks[1]	11.25		11.20		11.64		11.41	
Gross energy		16.56		16.84		16.48		16.81
Crude Fibre	3.15	5.43	3.18	5.56	2.87	3.96	2.92	4.13
Fat (Ether Extract)	4.87	6.50	5.22	7.35	5.46	5.95	5.45	6.57
Lysine[2]	1.23		1.35		1.00		1.25	
Methionine	0.56		0.62		0.43		0.58	
Total sulphur amino acids	0.83		0.92		0.70		0.88	
Threonine	0.76		0.84		0.64		0.78	
Tryptophan	0.21		0.23		0.19		0.22	
Arginine	1.27		1.40		1.16		1.30	
Isoleucine	0.81		0.89		0.75		0.85	
Valine	0.90		0.98		0.83		0.93	
Leucine	1.69		1.84		1.70		1.90	
Glycine and Serine	1.73		1.90		1.56		1.76	
C 18:2	2.66		2.74		2.92		3.04	
Calcium[3]	1.10	1.16	1.10	1.28	1.03	1.02	1.03	0.89
Potassium	0.94		1.00		0.94		1.00	
Chloride	0.20	0.25	0.20	0.25	0.20	0.25	0.20	0.27
Total Phosphorous	0.84	0.87	0.85	0.85	0.79	0.79	0.80	0.80
Retainable Phosphorous[4]	0.50		0.50		0.47		0.47	
Sodium	0.22	0.26	0.22	0.26	0.20	0.24	0.20	0.24

[1] OE for broiler chicks (CVB) [2] Amino acids availability for broiler chicks (CVB) for all amino acids [3] 0.08% Ca made available by addition of phytase enzyme[4] 0.08% P made available by addition of phytase enzyme [5] Additives: Pre-starter 0.025 kg contribution; Starter 0.125 kg contribution

Table 4-2 Raw material (ingredient) inclusion (%), calculated and analysed nutrient levels for the grower and finisher diets

Ingredients	**Grower (LP) Ingredient (%)**		**Grower (HP) Ingredient (%)**		**Finisher (LP) Ingredient (%)**		**Finisher (HP) Ingredient (%)**	
Maize	61.0		56.0		62.9		57.1	
Full fat soya (35.5%)	4.13		6.13		10.0		12.0	
Soybean oilcake (46%)	22.6		24.6		14.2		16.2	
Fish meal (65%)	-	-	-		1.00		2.00	
Sunflower oilcake (38%)	1.00		1.00		1.00		1.00	
Gluten (60%)	2.73		3.73		3.85		4.85	
Full fat maize germ	1.00		-		1.00		-	
L threonine	-		0.044		-		0.016	
DL methionine	0.094		0.231		0.039		0.155	
Lysine HCl	0.109		0.314		0.086		0.234	
Soya bean oil	3.39		4.10		2.56		3.23	
Salt	0.258		0.263		0.225		0.203	
Sodium bicarbonate	0.146		0.142		0.133		0.137	
Monocalcium phosphate	1.75		1.70		1.48		1.33	
Limestone	1.50		1.50		1.35		1.33	
Vitamin & minerals	0.177		0.177		0.167		0.167	
Nutrient Levels	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**	**Calculated**	**Analysed**
Dry matter	89.24	90.28	89.48	89.81	89.42	89.53	89.64	89.87
Crude protein	18.87	19.23	21.06	21.80	18.57	19.47	21.27	21.96
AME for chicks[1]	12.45		12.45		12.75		12.75	
Gross Energy		16.73		16.75		16.84		17.10
Crude fibre	2.66	5.61	2.71	5.18	2.73	3.38	2.77	4.71
Fat (Ether Extract)	7.26	7.33	7.76	7.58	7.67	7.41	8.24	8.03
Lysine[2]	0.85		1.10		0.79		1.04	
Methionine	0.37		0.53		0.33		0.48	
Total sulphur amino acids	0.62		0.80		0.58		0.76	
Threonine	0.58		0.68		0.57		0.67	
Tryptophan	0.17		0.19		0.16		0.19	
Arginine	1.06		1.17		1.00		1.14	
Isoleucine	0.69		0.77		0.67		0.77	
Valine	0.76		0.84		0.74		0.85	
Leucine	1.59		1.75		1.63		1.83	
Glycine and Serine	1.43		1.57		1.40		1.60	
C 18:2	2.92		3.04		3.26		3.35	
Calcium[3]	0.92	0.94	0.92	0.89	0.84	0.83	0.84	0.91
Potassium	0.91		0.96		0.79		0.85	
Chloride	0.20	0.20	0.20	0.27	0.20	0.21	0.20	0.24
Total Phosphorous	0.72	0.73	0.73	0.71	0.67	0.64	0.67	0.64
Retainable Phosphorous[4]	0.42		0.42		0.38		0.38	
Sodium	0.17	0.18	0.17	0.19	0.16	0.17	0.16	0.19

[1] OE for broiler chicks (CVB) [2] Amino acids availability for broiler chicks (CVB) for all amino acids [3] 0.08% Ca made available by addition of phytase enzyme[4] 0.08% P made available by addition of phytase enzyme [5] Additives: Grower 0.192 kg contribution; Finisher 0.092 kg contribution

a. Feed Analyses

Table 4-3 Calculated dietary available lysine (LYS SPL /kg feed) of the different treatments and phases

	LYS SPL/kg			
	Pre-starter	Starter	Grower	Finisher
Treatment				
A (100% Low Energy; LE)	1.15 LYS SPL	1.00 LYS SPL	0.85 LYS SPL	0.79 LYS SPL
B (80% LE:20% HE)	1.20 LYS SPL	1.05 LYS SPL	0.90 LYS SPL	0.84 LYS SPL
C (60% LE:40% HE)	1.25 LYS SPL	1.10 LYS SPL	0.95 LYS SPL	0.89 LYS SPL
D (40% LE:60% HE)	1.30 LYS SPL	1.15 LYS SPL	1.00 LYS SPL	0.94 LYS SPL
E (20% LE:80% HE)	1.35 LYS SPL	1.20 LYS SPL	1.05 LYS SPL	0.99 LYS SPL
F (100% High Energy; HE)	1.40 LYS SPL	1.25 LYS SPL	1.10 LYS SPL	1.04 LYS SPL

Lysine (LYS) Skynbare verteerbare amino sure pluimvee (SPL)

Representative samples of the different feeds were collected during the respective feeding phases, before the birds had access to the feed. Each sample was ground and analysed for dry matter (DM), ash, crude protein (CP), gross energy (GE), crude fibre (CF), ether extract (EE), calcium (Ca), phosphorus (P), sodium (Na) and chloride (Cl). The analysis was done at NutriLab (Department of Animal and Wildlife Sciences, University of Pretoria). Moisture was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 934.01). Dry matter and ashing were analysed according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 942.05). Crude protein was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 988.05). Gross energy was determined using the MC – 1000 Modular Calorimeter. Crude fibre was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 962.09). The Dosi fibre system was used to determine the crude fibre percentage. Crude fat was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 920.39).

Samples were prepared for calcium and sodium analysis following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 935.13). Samples were prepared for phosphorus analysis following the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 968.08.D.b.). Calcium was determined according to the Giron, H.C's official method of analysis (Giron. H.C., 1973, Perkin Elmer Atomic Spectrophotometer). Phosphorus was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 965.17). Sodium was determined according to the Giron, H.C's official method

of analysis (Giron. H.C., 1973, Perkin Elmer Atomic Spectrophotometer). Chloride was determined according to the AOAC's official method of analysis (AOAC, 2000, Official method of analysis 969.10).

4.3.3 Performance measurements

All live birds were weighed weekly on a per pen basis on day 7, 14, 21, 28 and 35 to calculate mean weekly body weights (BW) and weekly body weight gain. Remaining feed left in feeders of each pen were weighed back weekly as well as at the change of each feed phase (day 10, end of pre-starter; day 18, end of starter and day 28, end of the grower). Weekly feed intake (WFI), cumulative feed intake (CFI), weekly feed conversion ratio (FCR) and cumulative feed conversion ratio (CFCR) were calculated from the amount of feed provided and weighted back. Mortalities were collected twice daily, weighed and recorded; mortalities that were noted on a weighing day were recorded as a mortality on that day.

4.4 Statistical analyses

To assess the effect of energy on performance traits, a randomised block design was used to evaluate the data. The trial data was analysed as a mixed model using the Fit Model procedure with the REstricted Maximum Likelihood (REML) method. The fixed factors per house were Treatment (levels = 6) and House (levels = 2); and Block was included as a random factor (levels = 5). Although House could also be considered a random factor, it had to be included in the model as a fixed factor as there are only two levels.

The treatments in this trial were structured and therefore, simple analysis of treatment means was the most appropriate statistical analysis to be used on the data. Data were subjected to a statistical model and an analysis of variance (ANOVA) was done on all treatment means; where significant differences were indicated, post hoc multiple-comparison tests such as student t-test or Tukey's test were applied. The student t-test was an appropriate multi-comparison test for finding significant differences within a factor of 2 levels. However, the Tukey's test was an appropriate test to use when a factor has 3 or more levels within. The confidence level was set at 95%.

4.5 Results

In general, analysed values for DM, CP, GE, CF, EE, Ca, Cl, P and Na were close to the formulated values (Table 4-1; Table 4-2; Appendix: Table 4-10; Appendix: Table 4-11; Appendix: Table 4-12; Appendix: Table 4-13). Crude Protein analysed higher than the formulated value in all feeding phases for both Treatment A and F; with a concomitant increase in CP value from Treatment A to F throughout all manufactured diets. According to the data recorded on the day of placement (day 0), birds from various treatments did not differ significantly for body weight (BW) nor did BW at day 0 (BW0) contribute measurably at any stage ($P > 0.05$, Table 4-4).

Table 4-4 Mean weekly body weight (g) of mixed sex Hubbard Flex broilers fed increasing levels of dietary protein

	Body weight (g)					
	0 d	7 d	14 d	21 d	28 d	35 d
Treatment						
A (100% Low Protein; LP)	42.2	168.8	432.2^{c}	858.9^{c}	1375.7^{d}	1869.8^{c}
B (80% LP:20% HP)	42.2	172.1	443.6^{b}	883.1^{b}	1417.2^{c}	1921.3^{b}
C (60% LP:40% HP)	42.3	172.5	445.8^{ab}	888.9^{ab}	1417.6^{bc}	1914.8^{b}
D (40% LP:60% HP)	42.5	170.6	443.0^{b}	891.7^{ab}	1435.8^{abc}	1933.6^{ab}
E (20% LP:80% HP)	42.2	171.5	448.2^{ab}	898.8^{a}	1441.6^{ab}	1962.6^{a}
F (100% High Protein; HP)	42.1	173.0	451.7^{a}	901.0^{a}	1446.1^{a}	1937.5^{ab}
SEM	0.6	5.1	8.5	16.0	26.1	37.1
F-Prob:						
Treatment	0.870	0.503	0.000	0.000	0.000	0.000
Block	0.625	0.566	0.118	0.934	0.169	0.332
BW 0	-	0.715	0.433	0.566	0.115	0.091
R^2 (% Var)	0.52	0.29	0.57	0.59	0.68	0.68

^{a,b,c,d} Column means with common superscripts do not differ significantly ($P < 0.05$)

Broilers from HP were significantly heavier ($P < 0.001$) than broilers from LP from day 14 to day 35. On day 21, BW for broilers in Treatment F was 900.97 g compared to 858.85 g for Treatment A birds. This is also illustrated by Figure 4-1, displaying an almost linear increase in BW (R^2 = 0.94) at 21 days of age as the WPI increased during the grower phase. By day 35 Treatment F broilers weighed 1937.48 g compared to the 1869.80 g of Treatment A birds; however Treatment E birds weighed 1962.58 g ($P < 0.001$, Table 4-4) which was the heaviest at the end of the trial. Figure 4-2 shows the linear increase of BW (R^2 = 0.83) as well as WPI (R^2 = 0.96) as the dietary lysine in the finisher phase increases.

Average daily gain (ADG) showed similar trends to BW, where no significant difference was noticed until 14 days of age. Broilers in Treatment F had significantly higher ADG than Treatment

A from day 14 to day 28 (P < 0.001, Table 4-5). At day 21 Treatment F's ADG was 64.17 g/bird/d compared to 60.94 g/bird/d for Treatment A. However, Treatment A (70.55 g/bird/d) and F (70.21 g/bird/d) did not differ significantly from each other at day 35, but Treatment E (74.43 g/bird/d) displayed the fastest growth by day 35 (P > 0.01).

Table 4-5 Mean weekly body weight gain (g/bird/ d) of mixed sex Hubbard Flex broilers fed increasing levels of dietary protein

	Body weight gain (g/bird/ d)				
	0 - 7 d	7 - 14 d	14 - 21 d	21 - 28 d	28 - 35 d
Treatment					
A (100% Low Protein; LP)	15.8	37.6^{c}	60.9^{c}	73.8^{c}	70.6^{b}
B (80% LP:20% HP)	16.2	38.8^{b}	62.8^{b}	76.3^{ab}	72.0^{ab}
C (60% LP:40% HP)	16.3	39.1^{ab}	63.3^{ab}	75.7^{bc}	70.9^{b}
D (40% LP:60% HP)	16.0	38.9^{b}	64.1^{a}	78.0^{a}	71.3^{b}
E (20% LP:80% HP)	16.2	39.5^{ab}	64.4^{a}	77.5^{ab}	74.4^{a}
F (100% High Protein; HP)	16.4	39.8^{a}	64.2^{a}	77.7^{ab}	70.2^{b}
SEM	0.6	0.9	1.3	2.4	3.4
F-Prob:					
Treatment	0.453	0.000	0.000	0.000	0.009
Block	0.622	0.166	0.995	0.002	0.001
R^2 (% Var)	0.32	0.58	0.59	0.65	0.62

^{a,b,c} Column means with common superscripts do not differ significantly (P < 0.05)

In general, cumulative feed intake (CFI) decreased as the protein content of the feed increased. By day 21, broilers from Treatment A had a CFI of 1119.5 g/bird, compared to the 1089.93 g/bird for birds from Treatment F (P < 0.01, Table 4-6). Although the results from day 28 only approached significance, Treatment F consumed significantly less feed (2840.3 g/bird) than birds from Treatment A (2913.7 g/bird) by day 35 (P < 0.05, Table 4-6).

Cumulative feed conversion ratio (CFCR; cumulative feed intake: weigh gain ratio) displayed significant results throughout the experimental period. From day 7 to day 35, Treatment A and F differed nearly 10 points, with Treatment A having the highest value (P < 0.05). By day 21 the average CFCR value of broilers in Treatment A birds was 1.37 points compared to Treatment F's of 1.27; and by day 35, 1.59 points versus 1.50 for Treatment A and F, respectively (P < 0.001, Table 4-7).

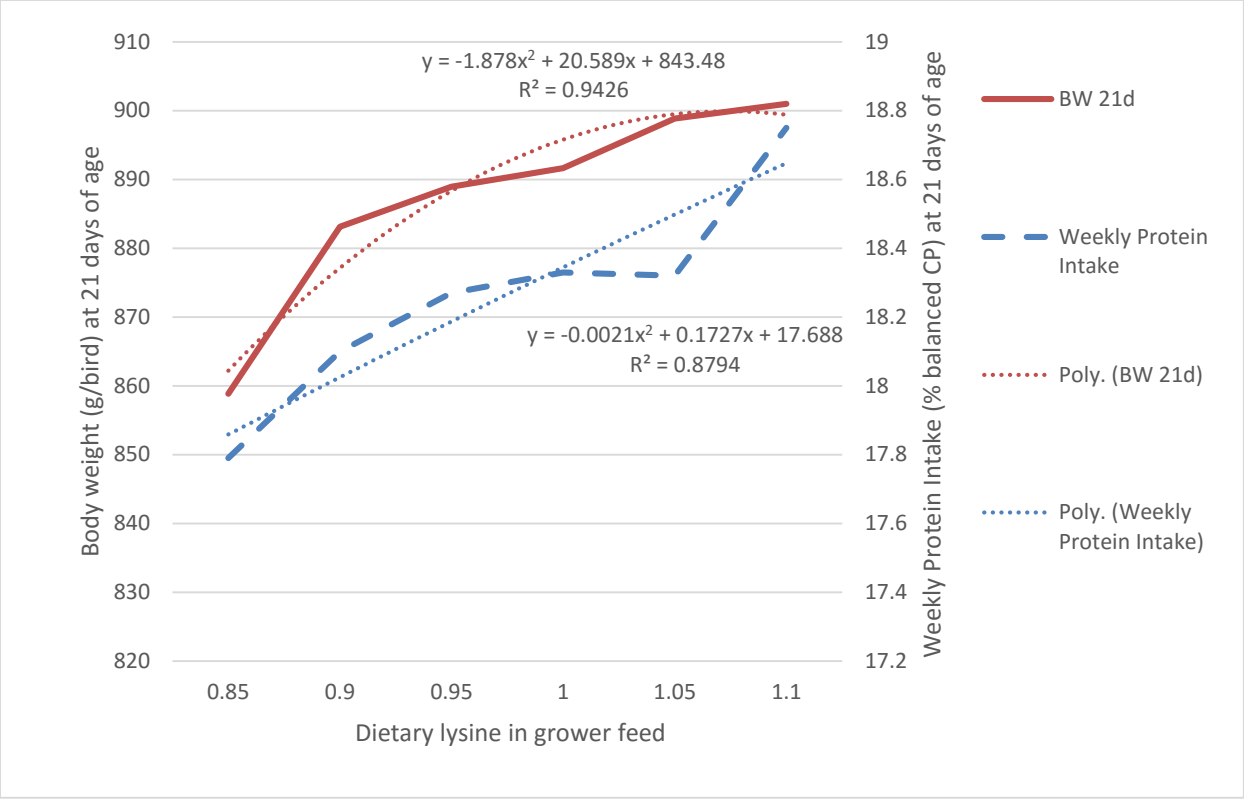

Figure 4-1 Body weight and weekly protein intake (- - -) (vertical bars) of mixed sex broiler chickens at day 21 of the experiment, as affected by increasing the dietary lysine in the grower from 0.85 to 1.10%. Polynomial trend line for BW – R^2 = 0.94; WPI – R^2 = 0.88

Table 4-6 Mean cumulative feed intake (g/bird) of mixed sex Hubbard Flex broilers fed increasing levels of dietary protein

	Cumulative feed intake (g/bird)				
	0 - 7 d	0 - 14 d	0 - 21 d	0 - 28 d	0 - 35 d
Treatment					
A - (100% Low Protein; LP)	139.4	500.8	1119.5^{c}	1960.3	2913.7^{c}
B - (80% LP:20% HP)	141.5	502.1	1117.6^{c}	1949.9	2908.6^{c}
C - (60% LP:40% HP)	140.5	503.3	1111.5^{bc}	1940.8	2884.1^{bc}
D - (40% LP:60% HP)	137.7	495.6	1101.2^{ab}	1936.5	2881.9^{bc}
E - (20% LP:80% HP)	137.8	495.4	1098.3^{ab}	1925.8	2870.6^{bc}
F - (100% High Protein; HP)	138.4	492.6	1089.9^{a}	1917.1	2840.3^{a}
SEM	3.8	11.3	17.1	32.5	48.1
F-Prob:					
Treatment	0.171	0.220	0.002	0.060	0.019
Block	0.422	0.506	0.420	0.445	0.731
R^2 (% Var)	0.36	0.39	0.47	0.41	0.52

^{a,b,c} Column means with common superscripts do not differ significantly (P< 0.05).

The increase in protein levels from Treatment A to F, did not cause a significant increase in mortalities (P > 0.05, Table 4-8) during any stage of the experiment; although a numerical increase could be seen from Treatment A to F with Treatment F having the highest mortality rate.

Weekly protein intake (WPI) increased numerically from Treatment A to F throughout the experimental period. Although WPI on day 21 did not differ significantly, by day 35 the WPI for Treatment A was 25.80% balanced CP compared to the 27.65% balanced CP of Treatment F (P < 0.05, Table 4-9).

Table 4-7 Mean cumulative feed conversion ratio (g feed/g gain) of mixed sex Hubbard Flex broilers fed increasing levels of dietary protein

	Cumulative feed conversion ratio (CFCR) (g feed/g gain)				
	0 - 7 d	0 - 14 d	0 - 21 d	0 - 28 d	0 - 35 d
Treatment					
A (100% Low Protein; LP)	1.10^{c}	1.29^{d}	1.37^{e}	1.47^{d}	1.59^{d}
B (80% LP:20% HP)	1.09^{bc}	1.25^{c}	1.33^{d}	1.42^{c}	1.55^{c}
C (60% LP:40% HP)	1.08^{abc}	1.25^{bc}	1.31^{cd}	1.41^{c}	1.54^{c}
D (40% LP:60% HP)	1.07^{ab}	1.24^{abc}	1.30^{bc}	1.39^{b}	1.52^{b}
E (20% LP:80% HP)	1.07^{ab}	1.22^{ab}	1.28^{ab}	1.38^{ab}	1.50^{a}
F (100% High Protein; HP)	1.06^{a}	1.20^{a}	1.27^{a}	1.37^{a}	1.50^{a}
SEM	0.03	0.03	0.02	0.02	0.02
F-Prob:					
Treatment	0.011	0.000	0.000	0.000	0.000
Block	0.039	0.050	0.146	0.293	0.132
R^2 (% Var)	0.66	0.68	0.83	0.88	0.89

^{a,b,c,d,e} Column means with common superscripts do not differ significantly (P < 0.05).

Table 4-8 Mean cumulative mortality (% birds placed) of mixed sex Hubbard Flex broilers fed increasing levels of dietary protein

	Cumulative mortality (% birds placed)				
	0 - 7 d	0 - 14 d	0 - 21 d	0 - 28 d	0 - 35 d
Treatment					
A (100% Low Protein; LP)	1.56	2.40	3.23	4.27	6.24
B (80% LP:20% HP)	1.88	2.92	3.65	4.79	7.60
C (60% LP:40% HP)	1.98	3.44	4.27	5.73	8.85
D (40% LP:60% HP)	2.19	3.65	4.49	6.07	9.41
E (20% LP:80% HP)	2.81	3.96	4.58	6.04	8.54
F (100% High Protein; HP)	1.98	3.86	4.59	5.94	9.06
SEM	1.80	2.04	2.28	2.77	3.26
F-Prob:					
Treatment	0.742	0.506	0.684	0,590	0.284
Block	0.016	0.007	0.055	0,274	0.910
R^2 (% Var)	0.42	0.45	0.37	0,30	0.25

Table 4-9 Mean weekly protein intake (%CP/bird/d) of mixed sex Hubbard Flex broilers fed increasing levels of dietary protein

	Weekly protein intake (% CP/bird/d)				
	0 - 7 d	7 - 14 d	14 - 21 d	21 - 28 d	28 - 35 d
Treatment					
A (100% Low Protein; LP)	3.95	11.12^{c}	17.79	23.03^{c}	25.80^{b}
B (80% LP:20% HP)	4.07	11.34^{bc}	18.10	23.26^{bc}	26.62^{ab}
C (60% LP:40% HP)	4.11	11.65^{ab}	18.27	23.65^{bc}	26.84^{ab}
D (40% LP:60% HP)	4.05	11.57^{ab}	18.33	23.99^{ab}	27.12^{a}
E (20% LP:80% HP)	4.07	11.61^{ab}	18.32	23.86^{abc}	27.26^{a}
F (100% High Protein; HP)	4.19	11.87^{a}	18.75	24.58^{a}	27.65^{a}
SEM					
F-Prob:					
Treatment	0.053	0.007	0.086	0.010	0.039
Block	0.859	0.710	0.998	0.381	0.609
R^2 (% Var)	0.37	0.51	0.39	0.49	0.49

^{a,b,c} Column means with common superscripts do not differ significantly ($P < 0.05$).

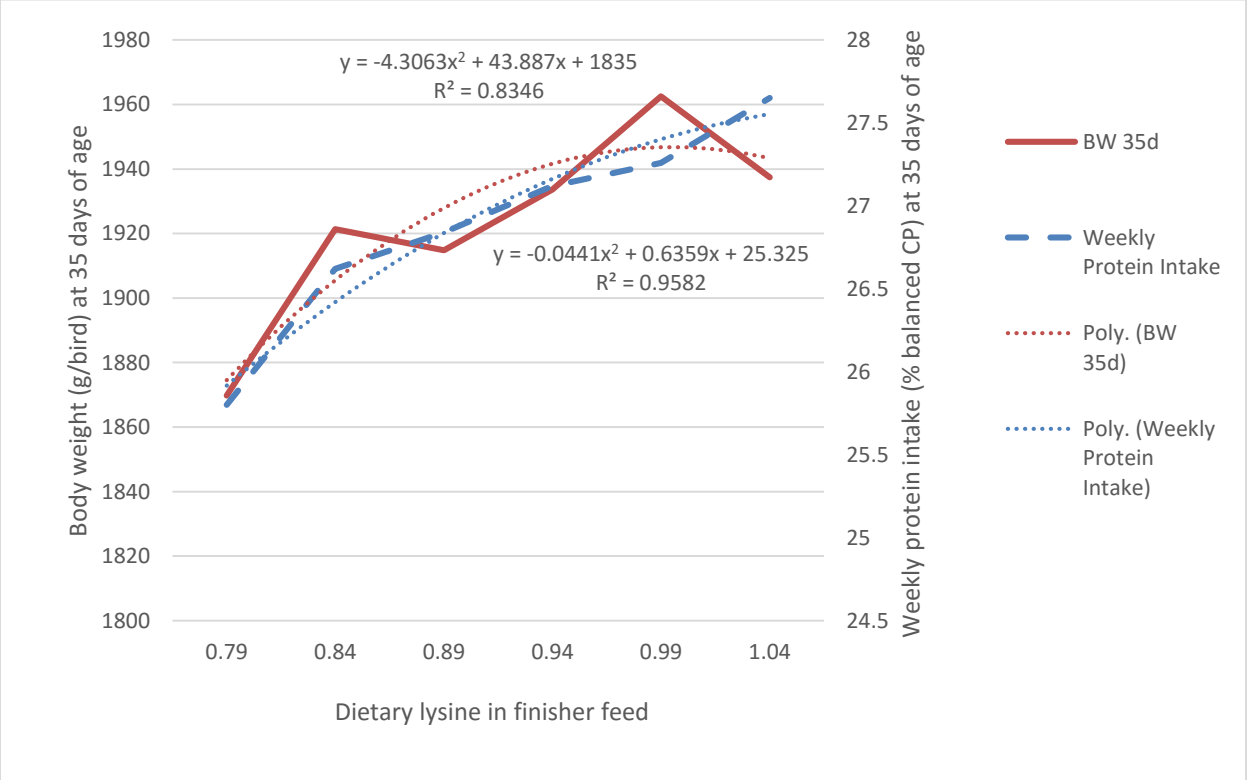

Figure 4-2 Body weight and weekly protein intake (- - -) (vertical bars) of mixed sex broiler chickens at day 35 of the experiment, as affected by increasing dietary lysine in the finisher from 0.79 to 1.04%. Polynomial trend line for BW – $R^2 = 0.83$; WPI – $R^2 = 0.96$

4.6 Discussion

The analysed nutrient levels were relatively close to calculated values. There were limitations with regard to the feed analysis i.e. dietary energy was analysed by means of measuring the GE, even though dietary energy was formulated on an AME (MJ/kg) basis.

The expected early linear increase in BW and BW gain were not observed as reported in previous research (Plumstead *et al.*, 2007). In fact, Treatment A (LP) and F (HP) only differed significantly from day 14 onwards. A more curvature response was noticed as the concentration of dietary Lys and balanced CP increased. The regression analysis showed in Figure 4-1 displays this significant curvature response at day 21, against the dietary Lys content of Treatment A to F during the grower phase (R^2 = 0.94). By day 21 both the starter and grower diets were fed to the birds; but since the dietary lysine levels increased at the same increment levels from Treatment A to F throughout the feeding phases, plotting BW against either starter or grower phase, provided the same curvature outcome for day 21. Although the response was not linear, the increase in BW was due to the increasing dietary lysine from Treatment A to F, also noticed by Plumstead *et al*. (2007). The early provision of higher dietary lysine and balanced CP showed that broilers respond during later growing phases; such effect is known as a carry-over effect found with growth rate and FCR (Eits *et al*., 2003). Broilers continued a significant increase in BW and BW gain from LP to HP diets until slaughter at day 35 (Table 4-4; Table 4-5, $P < 0.01$). This was also displayed in the Figure 4-2, continuing the almost linear increase in BW (R^2 = 0.83) at day35, with a concomitant increase in WPI (R^2 = 0.96) as the dietary Lys in the finisher phase increase.

Although neither breast meat yield nor carcass yield were measured in the present study, both contribute positively to BW. Berhe & Gous (2008) observed significant effects on breast meat yield when dietary CP alone increased as well as a considerable response on carcass yield when dietary CP level changed in relation to the AA profile. Therefore, by limiting AA to a restricted minimum in the dietary formulation and calculating the AA as a percentage of the dietary CP, an increase in dietary CP will cause a concomitant increase in the EAA levels; resulting in a positive response in breast meat yield as well as carcass yield.

Similarly for this study, Tesseraud *et al*. (2003) also found a reduction in BW and increased CFI for broilers that received diets with lower protein levels. This, contradicted the results of Plumstead *et al.* (2007), who reported no decrease in feed intake as dietary CP concentration

increased. Consequently, CFCR increased considerably for the LP diets. On the other hand, Smith & Pesti (1998) also reported a decrease in FI as dietary CP increased.

The dietary fat levels were higher in the HP diets for all feeding phases. These diets contained higher levels of lysine, achieved by inclusion of higher percentages of soybean oilcake as well as synthetic AA; other ingredients such as full fat soy beans, fish meal, maize gluten (60% CP), maize germ oil and lower levels of maize, contributed to higher levels of fats and oils (Table 4-1; Table 4-2). The higher levels of fat (Table 4-1; Table 4-2) in the HP diets may have had an effect on FI.

Tessesaud *et al*. (2003) measured the Insulin-like Growth Factor (IGF) I and II levels in the blood when differing dietary protein levels were given to broilers. Growth is a complex process, controlled by the interaction of genetic, hormonal and nutritional factors; and IGF are important positive modulators of body and muscle growth in chickens. The researchers found that higher levels of both IGF-I and IGF-II were secreted in birds fed high protein diets. The circulating concentrations of IGF-I and IGF-II were a result of the selection for an increased growth rate, higher breast muscle proportionate to the body tissue and reduced carcass fat, within fast growing genotypes.

CFCR observed was similar to the results in the study by Sterling *et al*. (2006); decreasing as dietary lysine increased up to the minimum level required for optimum performance (Leclercq, 1998). CFCR was significant among treatments and across the entire trial period. Similar results for FI, BW gain and CFCR were seen by Ferguson *et al*. (1998) where a decrease in CP levels caused for greater feed efficiency. Birds fed medium protein treatments consumed 6% more feed / unit of body weight gain compared to birds fed HP treatments; showing that increased protein levels improved performance efficiency, whether utilising or producing body tissue, especially breast meat yield. Similar findings were mentioned by Vieira & Angel (2012), where it was demonstrated that dietary CP provided at concentrations above that used by the US industry lead to improved FCR and breast meat yield.

Pesti (2009) stated that the CP level was a major contributing factor to variation in broiler growth and FCR. Since modern broiler strains grow fast and yield high amounts of lean meat (Berhe & Gous, 2008), they require greater amounts of daily protein. Genetic selection for these traits have imposed improved efficiency in utilising dietary protein as well as greater rates of protein

deposition (Morris & Njuru, 1990). With this said, chickens fed LP diets, often fail to perform efficiently and are excessively fat (Pesti, 2009).

The increases in BW as either muscle tissue and/or body fat is speculative, since no carcass analysis was done in this study; but due to genetic selection of leaner carcasses with greater muscle tissue, the expected outcome would be for the breast meat yield to be improved on HP-diets. This can be supported by Tesseraud *et al*. (2003) who found that dietary treatment had a more pronounced effect on breast muscle, compared to leg muscle. As discussed by Teeter *et al*. (1996), genetic selection for fast growing broiler lines exhibit an increase in the number (20%) and size (90%) of muscle fibres as well as the total amount of protein gained in the *pectoralis major* muscle. Smith *et al*. (1998) found a significant linear reduction in both the rate and efficiency of gain when dietary PL were dropped from 24% to 16% CP. However, not all researchers found that differing PL affected the BW gain (Ferguson *et al*., 1998), FI or FCR (Summers *et al*., 1992).

No significant difference was found for mortality between any of the treatments, although a numerical increase was noticeable from Treatment A to F. These results agree with results from Eits *et al*. (2003) who reported that feeding broilers a diet low in first limiting AA to energy ratio during the early growth stage (day 14 – day 21) has the potential to lower the incidence of metabolic disorders at later stages.

As for weekly protein intake (WPI), broilers from Treatment F consumed greater amounts of dietary protein at equal FI compared to Treatment A birds due to elevated PL from Treatment A to Treatment F. Present research findings confirm that the increase dietary lysine increased body weight throughout a broiler cycle. Genetic selection which has allowed the broiler chickens to display a high allometric growth rate for breast muscle, have led to a high requirement for balanced AA. Diets providing required and balanced AA, result in continuous growth in modern broiler birds (Vieira & Angle, 2012).

4.7 Conclusion

Maximum performance is reached when broiler response plateaus, whereas an optimal response occurs when the highest return per input is achieved (Vieira & Angel, 2012). When selecting an optimal diet with protein (CP or as a percentage of lysine) as the intentional first limiting nutrients,

it is beneficial to save feed production costs and improve the carcass quality; especially since increase in PL decrease carcass fat and increase breast meat yield (Tesseraud *et al.,* 2003). In this study, broiler performance responded positively by increasing dietary CP with concomitant reduction in CFI. BW and ADG were higher in broilers fed higher levels of dietary CP. Although broilers from Treatment E had the highest BW and ADG by 35 days of age, in comparison to broilers fed HP diets, the broilers from Treatment F displayed an overall greater performance than broilers fed the LP diet. BW, ADG, CFI and CFCR were improved in broilers fed HP diets.

Defining AA requirements and levels required for performance throughout the life cycle of a modern commercial broiler strain is imperative to optimise marketable meat in a profitable manner (Eits *et al*., 2003). To optimise these economic returns, dietary AA levels should be adapted to the specific strain of broiler and the particular physiological stage of growth in the broiler's life (Vieira & Angel, 2012).

Supplying the correct amount of AA for each commercial strain may reduce dietary costs. Too low levels of AA may cause suboptimal growth and meat yield and ultimately negate economic returns, while excess AA is costly without realising any additional returns on protein mass and may even have detrimental effects such as protein catabolism and reduced intake with concomitant poor growth (Kidd *et al*., 2004).

In the past, general feeding practice was to provide broilers with high AA concentration during early feeding phases, resulting in greater economic returns due to the relatively low FI and high growth rate, as well as an apparently carry-over effect to later growth. However, increasing dietary lysine and other EAA until the finisher diet has shown to improve FCR and BW significantly. This is due to the positive allometric growth of muscle compared to other body tissues that is of great advantage at later stages of broilers' development (Vieira & Angel, 2012). In the present study, the increase in BW and the reduction in CFI for broiler from LP to HP treatments, led to a positive response in CFCR, with an overall sparing of 9 points between broilers from LP to HP by 35 days of age. This, however, also resulted in a higher percentage mortality, although the finding were not significantly different between broilers fed LP diets compared to birds fed HP diets.

GENERAL CONCLUSION

In commercial broiler practice, the nutrient density of diets will be limited by the associated exponential increase in dietary costs as the concentration of dietary CP or ME increases. The nutrient recommendations for broilers should be sufficient to allow for a positive response to the diet; for example, daily growth and body weight gain as well as an efficient utilisation of the feed. However, the dietary ME, CP and AA concentrations which optimise broiler performance may differ depending on the production target of the operation. Increased concentrations of TSAA may cause greater returns for breast meat yield compared to overall BW or carcass yield, depending on broiler genetics. When considering the amount of synthetic AA supplementation, factors such as the desired market weight, live broiler cost, mixed product requirement and genetics should be considered.

The difference in response between increased dietary energy compared to dietary lysine was clearly noticeable in the performance of the broilers in each current experiment. These finding were in agreement with Sterling *et al*. (2006), acknowledging that the rapid growth rate of the present-day broilers requires increased amounts of all nutrients on a daily basis, including energy. Due to continuous genetic selection the improvements in nutritional demands changed and AA requirements increased proportionally faster than energy requirements. Thus, a higher AA to energy ratio is required in faster growing broiler strains, with a great effect noticed when dietary AA increases albeit smaller increments compared to dietary energy.

Hubbard Flex broilers did not respond significantly at low incremental levels of dietary ME with a concomitant constant dietary CP level. It was concluded that the increments between the energy levels in the different treatments in trial 1 was not sufficient to cause a significant difference in performance attributes such as body weight and average daily gain ($P > 0.05$).

Experimental data from Experiment 2 showed an improvement in FCR and BW with increased dietary CP at a constant dietary ME level. Higher AA concentrations showed greater returns, especially during the later stages of the protein dose-response trial. These results confirmed the findings of Vieira & Angel (2012). According to Vieira & Angel (2012), the improvement in CFCR and BW with increased dietary Lys could be attributed to positive allometric growth of breast muscles compared to other body tissues at later ages. The positive growth in breast muscle contributes considerably to the overall BW of a broiler since it displays the highest value of growth

of all the portions and body tissue. Although carcass analysis were not done on broilers from either experiment, high BW at higher dietary protein confirmed this response.

Leeson *et al*. (1996) noticed a considerable decrease in feed intake when dietary ME was increased from 11.15 MJ to 13.62 MJ/kg. In the present study AME was only increased from 12.15 MJ/kg to 12.95 MJ/kg during the finisher phase. No significant effects for FI ($P > 0.05$) were noted with the increasing dietary ME and it was concluded that the incremental differences among treatments were too small to have an effect on FI. Plumstead *et al.* (2007) also noticed no effect on FI between different levels to ME for broilers. As a consequence of the lack of response in FI from Treatment A to F weekly energy intake (WEI) increased significantly at all weekly recordings ($P < 0.001$), as dietary EL increased. This did not have a significant effect on weekly mortality in birds fed the Treatment F diet ($P > 0.05$).

In the study by Leeson *et al*. (1996) dietary energy was increased by adding an animal-vegetable fat blend between 1.15% to 8.65% in order to achieve the large ME difference between treatments; compared to the 2% of added soy bean oil in Experiment 1. The larger quantity of added oil/fat in the experiment of Leeson *et al*. (1996) could have had a compounding effect on FI, meaning that lipid level and ME level both affected FI. Formulation is therefore important to prevent the excessive addition of fats and the proportion of dietary ME derived from added fat.

Linear least-cost feed formulation software is widely used to provide economic solutions for all nutrients with set specifications (minimum and maximum inclusion levels). Differences exist between the formulation approaches by nutritionists, especially with regard to protein in feeds; ultimately affecting the concentration and balance of the formulated AA. This in turn will have a potential effect on the ability of the broiler to express its capacity for growth and muscle deposition. To overcome this, nutritionists define a minimum CP level during feed formulation, providing a safety margin for maintaining minimum EAA as well as NEAA concentrations without defining specific levels for each. Diets should still be formulated to the ideal protein content, since it allows for greater precision in formulation and ensures a balanced AA profile that optimises performance and yields (Vieira & Angel, 2012). From this experiment, increasing dietary CP and AA were achieved by increasing high protein raw materials as well as ingredients with higher fat content. CFI lowered as the dietary protein increased, a result not found by Plumstead *et al.* (2007) who concluded that an increased dietary lysine or ME had no effect on feed intake. The increased ME

in Experiment 1 was achieved by adding vegetable oil to high energy treatments and no effect was noticed on feed intake; as in agreement with Plumstead *et al.* (2007).

Imbalances between nutrients, especially energy to protein ratio and the imbalance between the first and/or second-limiting AA, cause metabolic inefficiencies. Dietary CP and AA requirements should be expressed as a percentage of the dietary ME concentration and the increase in dietary CP without considering the increase in ME or dietary fat may greatly influence the response to the increased dietary CP and AA. ME and dietary fat increased in order to achieve higher dietary CP and AA; an effect that might have contributed towards the response noticed in Experiment 2.

Plumstead *et al*. (2007) concluded that the response in BW gain and FCR from balanced CP of 27.2% (1.32% lysine) was independent of the dietary ME level over a range of 12.55 MJ ME/kg to 13.40 MJ ME/kg. When dietary Lys requirements were are expressed as a percentage of the dietary CP, a linear response in broiler performance was shown to be independent of the dietary ME concentrations within a particular range. The early provision of higher dietary lysine and balanced CP showed that broilers respond during later growing phases; such effect is known as a carry-over effect found with growth rate and FCR (Eits *et al*., 2003). In trial 2, Hubbard Felx broilers continued a significant increase in BW and BW gain from LP to HP diets until slaughter at day 35 ($P < 0.01$). Cumulative feed conversion ratio (CFCR; cumulative feed intake: weigh gain ratio) displayed significant results throughout trial 2. From day 7 to day 35, Treatment A and F differed nearly 10 points, with Treatment A having the highest value ($P < 0.05$). By day 21 the average CFCR value of broilers in Treatment A birds was 1.37 points compared to Treatment F's of 1.27; and by day 35, 1.59 points versus 1.50 for Treatment A and F, respectively ($P < 0.001$).

In conclusion, the differing energy levels from Exp. 1 did not affect the FI of Hubbard Flex broilers significantly and although an effect in BW was noticed, this was not significant. The differing protein levels did however have a significant impact in FI of the Hubbard Flex broilers, as well as cause a significant effect in BW and average daily gain during specific feed phases. Information from these two experiments can be deducted in further studies evaluating the Hubbard Flex breed.

CRITICAL REVIEW AND RECOMMENDATION

For this study, feed was manufactured in a commercial operation which mixed batches of 4 ton each. Therefore, a small scale feed operation would have provided a higher probability of manufacturing batches, more accurately. As a result of the large feed batches, only portion of the batch was used for the trial diets; either for the basal diets or for further dilution into the blended feed treatments. The remainder of the feed was discarded and this allowed possible variation within the dietary treatments. Ideally, all manufactured feed should be used within the experiment to reduce the variation brought on by differing nutrient values of raw materials within a single batch of feed.

The addition of soy bean oil was to increase the energy content of the feed (Exp. 1) subsequently, creating possible variation in broiler performance, attributed to the effect of the vegetable oil. To prevent the use of added oils or fats, differentiation within dietary treatments, in energy or protein, should be achieved by using varying levels of raw materials which are consistent in all treatments of the experiment.

Analysis of the AA of the feed would have been favourable since AA is expressed in proportion to the dietary CP and was a reference with regards to nutrient content of the feed. Formulation is based on both dietary CP and AA and thereby only analysing dietary CP depicts only one side of the actual outcome.

Numerous studies have been published to demonstrate the difference in performance between males and females due to their difference in maintenance requirements. Variation among factors influencing feed efficiency such as: growth rate, the shape of the growth curve and carcass fat percentage are also measurable between sexes. Female broilers have a lower basal metabolism rate and for this reason, we recognise the possibility of different nutritional requirements. Generally, a common diet would be fed to both sexes and when an average weight-to-age at which birds can be marketed is reached, the dietary specifications are accepted. Under severe and challenging conditions such as high temperatures, males' body weight gain is affected more adversely than females, and feed efficiency is better for females. Under these conditions it will be advantageous to provide sex specific diets. In the present study, sexing of the broilers were not applied. In order to determine a more accurate nutrient requirement within a dose-response trail, the same diet should be fed separately to male and female. This would have provided the

opportunity to determine effect of varying dietary ME and CP levels between sexes. This said, the initial cost implications would have been higher for a sex specific trial, since birds would have had to be sexed during or before placement. Thin different sexes would have also had to be accommodate separately in the houses; ultimately higher costs for handling and equipment would have been required. The plausible effect of sex specific diets on the carcasses of males and females may have allowed for more target specific final products; as well as the manipulation of carcass fat by changing the energy: protein (CP and AA) for each sex.

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APPENDIXES

Table 3-10 Nutrient analysis of pre-starter diet for Exp. 1

	Treatment A (LE)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HE)
Dry Matter (DM %)	88.8	91.0	91.0	90.8	90.0	90.7
Crude Protein (CP %)	25.9	25.9	25.8	25.5	24.6	24.7
Gross Energy (GE)	16.6	16.6	16.7	17.0	17.0	17.1
Crude Fibre (CF %)	4.8	5.6	4.6	5.6	5.9	5.1
Ether Extract (EE %)	5.4	5.9	6.2	6.5	6.9	7.1
Calcium (Ca %)	1.3	1.3	1.2	1.2	1.1	1.1
Chloride (Cl mg/kg)	2.3	2.1	2.2	2.1	2.2	2.2
Phosphorus (P % of Total P)	0.86	0.86	0.84	0.83	0.81	0.82
Sodium (Na %)	0.22	0.23	0.23	0.23	0.21	0.22

LE – Low Energy diet; HE – High Energy diet

Table 3-11 Nutrient analysis of starter diet for Exp. 1

	Treatment A (LE)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HE)
Dry Matter (DM %)	90.8	91.1	91.0	90.4	90.4	90.7
Crude Protein (CP %)	23.2	23.2	23.3	23.5	23.3	23.3
Gross Energy (GE)	16.3	16.4	16.6	16.6	16.7	16.9
Crude Fibre (CF %)	4.3	4.2	4.5	4.4	5.0	5.7
Ether Extract (EE %)	5.4	5.9	6.5	6,7	7.0	7.6
Calcium (Ca %)	1.2	1.2	1.2	1.3	1.2	1.2
Chloride (Cl mg/kg)	2.1	2.1	2.1	2.1	2.1	2.0
Phosphorus (P % of Total P)	0.83	0.83	0.80	0.83	0.82	0.79
Sodium (Na %)	0.20	0.21	0.19	0.19	0.17	0.17

LE – Low Energy diet; HE – High Energy diet

Table 3-12 Nutrient analysis of grower diet for Exp. 1

	Treatment A (LE)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HE)
Dry Matter (DM %)	89.9	89.4	90.3	90.6	90.8	91.0
Crude Protein (CP %)	19.7	19.6	19.7	19.6	19.8	19.4
Gross Energy (GE)	16.2	16.1	16.3	16.5	16.8	16.6
Crude Fibre (CF %)	3.7	3.8	4.4	3.9	4.5	3.9
Ether Extract (EE %)	4.8	5.1	5.6	5.9	6.4	6.8
Calcium (Ca %)	0.9	1.0	1.0	1.0	1.0	1.0
Chloride (Cl mg/kg)	2.5	2.8	2.5	2.6	2.5	2.5
Phosphorus (P % of Total P)	0.69	0.72	0.69	0.71	0.70	0.67
Sodium (Na %)	0.13	0.14	0.14	0.14	0.14	0.13

LE – Low Energy diet; HE – High Energy diet

Table 3-13 Nutrient analysis of finisher diet for Exp. 1

	Treatment A (LE)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HE)
Dry Matter (DM %)	89.4	89.5	90.0	89.7	89.9	89.4
Crude Protein (CP %)	19.6	19.5	19.7	19.4	19.5	19.3
Gross Energy (GE)	16.3	16.7	16.7	16.7	16.9	16.9
Crude Fibre (CF %)	3.5	4.6	4.1	4.1	5.4	3.6
Ether Extract (EE %)	4.8	5.1	5.6	5.9	6.4	6.8
Calcium (Ca %)	0.9	0.9	0.9	0.8	0.8	0.8
Chloride (Cl mg/kg)	2.8	2.6	2.6	2.5	2.4	2.3
Phosphorus (P % of Total P)	0.64	0.63	0.64	0.62	0.63	0.63
Sodium (Na %)	0.15	0.14	0.15	0.15	0.14	0.15

LE – Low Energy diet; HE – High Energy diet

Table 3-14 Temperature profile for Experiment 1 and Experiment 2

	Temperature (ºC, 50 % rH)		
Day	Lower Temp	**Target Temp**	Upper Temp
-1	34.0	**35.5**	37.0
-2	34.0	**35.5**	37.0
0	34.0	**35.5**	37.0
1	34.0	**35.5**	37.0
2	34.0	**35.5**	37.0
3	33.0	**34.5**	36.0
4	33.0	**34.5**	36.0
5	33.0	**34.5**	36.0
6	32.0	**33.5**	35.0
7	32.0	**33.5**	35.0
8	32.0	**33.5**	35.0
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	**25.0**	26.5
19	23.5	**25.0**	26.5
20	23.5	**25.0**	26.5
21	22.5	**24.0**	25.5
22	22.5	**24.0**	25.5
23	22.5	**24.0**	25.5
24	21.5	**23.0**	24.5
25	21.5	**23.0**	24.5
26	21.5	**23.0**	24.5
27	21.5	**23.0**	24.5
28	21.5	**23.0**	24.5
29	21.5	**23.0**	24.5
30	21.5	**23.0**	24.5
31	21.5	**23.0**	24.5
32	21.5	**23.0**	24.5
33	21.5	**23.0**	24.5
34	21.5	**23.0**	24.5
35	21.5	**23.0**	24.5

Table 3-15 Lighting program for Experiment 1 and Experiment 2

	Controller set point			
Day	**Lights on**	**Lights off**	**Day Light**	**Darkness**
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	23:00	23:00	01:00
5	00:00	23:00	23:00	01:00
6	00:00	23:00	23:00	01:00
7	05:00	19:00	14:00	10:00
8	05:00	19:00	14:00	10:00
9	05:00	19:00	14:00	10:00
10	05:00	19:00	14:00	10:00
11	05:00	19:00	14:00	10:00
12	05:00	19:00	14:00	10:00
13	05:00	19:00	14:00	10:00
14	05:00	19:00	14:00	10:00
15	05:00	19:00	14:00	10:00
16	04:00	20:00	16:00	08:00
17	04:00	20:00	16:00	08:00
18	04:00	20:00	16:00	08:00
19	04:00	20:00	16:00	08:00
20	04:00	20:00	16:00	08:00
21	04:00	20:00	16:00	08:00
22	04:00	20:00	16:00	08:00
23	03:00	21:00	18:00	06:00
24	03:00	21:00	18:00	06:00
25	03:00	21:00	18:00	06:00
26	03:00	21:00	18:00	06:00
27	03:00	21:00	18:00	06:00
28	03:00	21:00	18:00	06:00
29	02:00	22:00	20:00	04:00
30	02:00	22:00	20:00	04:00
31	02:00	22:00	20:00	04:00
32	02:00	22:00	20:00	04:00
33	02:00	22:00	20:00	04:00
34	02:00	22:00	20:00	04:00
35	02:00	22:00	20:00	04:00

d

Table 4-10 Nutrient analysis of pre-starter diet for Exp. 2

	Treatment A (LP)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HP)
Dry Matter (DM %)	90.8	91.1	91.0	90.4	90.4	90.7
Crude Protein (CP %)	23.2	23.2	23.3	23.5	23.3	23.3
Gross Energy (GE)	16.3	16.4	16.6	16.6	16.7	16.9
Crude Fibre (CF %)	4.3	4.2	4.5	4.4	5.0	5.7
Ether Extract (EE %)	5.4	5.9	6.5	6.7	7.0	7.6
Calcium (Ca %)	1.2	1.2	1.2	1.3	1.2	1.2
Chloride (Cl mg/kg)	2.1	2.1	2.1	2.1	2.1	2.0
Phosphorus (P % of Total P)	0.83	0.83	0.80	0.83	0.82	0.79
Sodium (Na %)	0.20	0.21	0.19	0.19	0.17	0.17

LP – Low Protein diet; HP – High Protein diet

Table 4-11 Nutrient analysis of starter diet for Exp. 2

	Treatment A (LP)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HP)
Dry Matter (DM %)	90.7	90.5	91.0	90.5	90.6	90.7
Crude Protein (CP %)	21.7	22.0	22.7	22.9	23.7	24.2
Gross Energy (GE)	16.5	16.6	16.7	16.6	16.8	16.8
Crude Fibre (CF %)	4.0	4.1	4.3	4.5	4.6	4.1
Ether Extract (EE %)	6.0	6.2	6.1	6.2	6.5	6.6
Calcium (Ca %)	1.0	1.0	1.0	1.0	0.9	0.9
Chloride (Cl mg/kg)	2.5	2.5	2.8	2.7	2.6	2.7
Phosphorus (P % of Total P)	0.79	0.79	0.81	0.82	0.82	0.80
Sodium (Na %)	0.24	0.23	0.24	0.24	0.23	0.24

LP – Low Protein diet; HP – High Protein diet

Table 4-12 Nutrient analysis of grower diet for Exp. 2

	Treatment A (LP)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HP)
Dry Matter (DM %)	90.3	90.3	90.5	90.1	90.2	89.8
Crude Protein (CP %)	19.2	19.9	20.0	21.0	21.0	21.8
Gross Energy (GE)	16.7	16.7	16.9	16.8	16.9	16.8
Crude Fibre (CF %)	5.6	4.7	5.3	4.9	5.2	5.2
Ether Extract (EE %)	7.3	7.4	7.9	7.5	7.9	7.6
Calcium (Ca %)	0.9	1.0	1.0	0.9	0.9	0.9
Chloride (Cl mg/kg)	2.0	2.2	2.4	2.3	2.5	2.7
Phosphorus (P % of Total P)	0.73	0.74	0.74	0.73	0.72	0.71
Sodium (Na %)	0.18	0.18	0.19	0.19	0.18	0.19

LP – Low Protein diet; HP – High Protein diet

Table 4-13 Nutrient analysis of finisher diet for Exp. 2

	Treatment A (LP)	Treatment B	Treatment C	Treatment D	Treatment E	Treatment F (HP)
Dry Matter (DM %)	89.5	90.0	89.9	90.1	89.7	89.9
Crude Protein (CP %)	19.5	20.0	20.5	21.4	21.1	22.0
Gross Energy (GE)	16.8	16.9	17.0	17.0	17.1	17.1
Crude Fibre (CF %)	3.4	5.2	5.5	4.8	4.2	4.7
Ether Extract (EE %)	7.4	7.7	7.8	7.8	7.8	8.0
Calcium (Ca %)	0.8	0.9	0.8	0.9	0.8	0.9
Chloride (Cl mg/kg)	2.1	2.1	2.3	2.3	2.4	2.4
Phosphorus (P % of Total P)	0.64	0.64	0.64	0.64	0.63	0.64
Sodium (Na %)	0.17	0.17	0.18	0.17	0.18	0.19

LP – Low Protein diet; HP – High Protein diet