The Effect of Varying Dietary Nutrient Densities on Performance: Experimental Investigations on the Response of Broiler Chickens to Different Energy and Lysine levels

By

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Abstract

The overall objective of the study was to determine the influence of varying dietary apparent metabolizable energy (AMEn) and digestible lysine (dLys) inclusion levels on the overall growth performance of Cobb 500 broilers. Two experiments were conducted, and in each experiment a total of 1440 unsexed day-old Cobb 500 broiler chicks were randomly allocated to one of 48 pens (i.e. 30 chicks per pen) in a completely randomized block design. In experiment 1, the effect of incremental changes in dietary AMEn while maintaining a constant dLys: AMEn ratio was assessed. Experimental diets were formulated to contain eight different dietary AMEn concentrations ranging from 11.30 to 13.05 MJ/kg (+0.25MJ/kg) respectively, while adjusting dLys levels to maintain a constant dLys: AMEn ratio of 0.97. The broilers received a common broiler started diet for a period of 14 days, after which the experimental diets were introduced (Day 14-35 of the trial). Feed intake decreased linearly with incremental changes in dietary AMEn. Dietary AMEn intake and energy efficiency ratio (EER) differed significantly between treatments. Dietary AMEn increased linearly with incremental changes in AMEn, while EER exhibited a quadratic response. No significant differences in body weight gain (BWG) were observed for the entire treatment period (Day 14-35). According to the response curve equation ($Y = -58.952x^2 + 1473.9 - 6937.9$), BWG was optimised at 12.55 MJ/kg. Increasing AMEn while maintaining the dLys: AMEn ratio improved feed conversion ratio (FCR), and significant differences occurred between the lowest and the highest nutrient densities.

In experiment 2, the influence of varying dietary dLys levels in isoenergetic diets was evaluated. Experimental diets were formulated to contain eight different dietary dLys levels ranging from 0.95 to 1.30% (+0.05%). Dietary AMEn concentration was kept constant at 12.1 MJ/kg giving dLys: AMEn ratios 0.78, 0.83, 0.87, 0.91, 0.95, 0.99, 1.03 and 1.07. The broilers were fed a common broiler started diet for the first 14days, whereafter the experimental diets were fed from Day 14-35 of Experiment 2. Feed intake and AMEn intake were not affected by the incremental changes in dietary dLys. Energy efficiency ratio, dLys intake and digestible lysine ratio (dLysER) differed significantly between dietary treatments. Digestible Lys intake increased, while dLysER decreased with an increase in dLys inclusion level. Overall, BWG differed significantly between treatment groups, with an increasing quadratic response ($Y = -1119x^2 + 2871.7x + 301.11, P < 0.05$). Feed conversion ratio decreased (improved) linearly with increasing dLys levels. It can be concluded that broilers can sustain optimal growth performance in a range of dietary AMEn concentrations, provided that an ideal balance between dietary AMEn and nutrients is maintained. Furthermore, adjusting the dLys: AMEn ratio when feeding one dietary AMEn concentration for growing and finishing will optimise broiler performance.

Keywords: broiler, digestible lysine, dietary energy, apparent metabolizable energy, energy concentration; digestible lysine: energy ratio; performance

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Table of Contents

List of Tables

List of Figures

List of abbreviations

AA	Amino acid
AME	Apparent metabolizable energy
AMEn	Nitrogen corrected apparent metabolizable energy
BW	Body weight
BWG	Body weight gain
CP	Crude protein
dLys	Digestible lysine
dLys ER	Digestible lysine efficiency ratio
EAA	Essential AA
EER	Energy efficiency ratio
FI	Feed intake
FCR	Feed conversion ratio
Lys	Lysine
ME	Metabolizable energy
N	Nitrogen

Chapter 1

General Introduction

Broilers are a significant component of the livestock industry and the primary source of animal protein for human consumption. Research shows that since the 1960s, poultry meat production has grown more rapidly than any other meat type in developed and developing countries (Nkukwana, 2018). It is projected that this growth trend will likely continue because modern broilers are fast-growing and feed-efficient, at least to a fair extent, considering the influence of other related factors such as dietary nutrient composition, housing conditions and stocking density (Simsek *et al.*, 2009). Siegel (2014) indicated that in 1985, around 3.22 kg of broiler feed was required to achieve a 1.40 kg live weight at 35 days of age, resulting in a 2.3 feed conversion ratio (FCR). In comparison, 3.66 kg of feed was needed to achieve 2.44 kg within the same growth period, resulting in a 1.5 FCR two and a half decades later (Siegel, 2014).

Nevertheless, modern broilers are the subject of continuous efforts to increase feed efficiency, motivated by the demand for economical and sustainable production. Multiple studies indicate that modern broilers may require higher dietary nutrient concentrations for optimum performance in contrast to earlier strains (Siqueira *et al.*, 2013; Bernal *et al.*, 2014), even though they consume significantly less feed overall compared to the latter to reach market weight (Siegel, 2014). At the same time, some publications have reported non-linear broiler responses to increased nutrient concentrations (Acacio *et al.*, 2020), while some even recommend levels below the National Research Council (NRC, 1994) values (Wang *et al.*, 2016; Belloir *et al.*, 2017; Lee *et al.*, 2020). Therefore, despite these characteristic advances, there is still a necessity for more precision in diet formulation and feeding strategies if the added benefit of genetic improvement in broiler chickens is to be realised (Moss *et al.*, 2021).

Research-driven-strategic industry practices have collectively facilitated the broiler industry's growth and success. One such strategy is diet optimization, choosing the most suitable combination and quantity of feed ingredients to meet the broiler's specific nutritional needs while considering factors such as production goals such increasing breast yield and while managing feed costs. Dietary energy and protein (i.e. lysine) thus are key nutrients to consider, and achieving an optimal balance is crucial to the broiler’s growth and feed efficiency. Gous *et al.* (2018) successfully demonstrated that the ratio of a feed's apparent metabolizable energy (AMEn) to digestible crude protein (DCP) determines protein utilisation efficiency (e_p), independently of the broiler’s sex, dietary protein quality (balanced vs unbalanced), and feed allocation (i.e. *ad libitum* vs restricted). Overall, the study of Gous *et al.* (2018) showed that e_p is a linear–plateau function with a critical point of about 66.2 ± 1.98, below which e_p declines; the values ranged between 38.5 and 85.5 MJ AMEn/kg DCP.

As researchers explore the complex interplay between dietary energy and protein concentrations, it becomes apparent that careful adjustments in these diet parameters can have profound effects on broiler performance. For example, Strifler *et al.* (2023) evaluated the effect of feeding low protein (LP) diets with different energy-to-protein ratios on broiler growth performance. The diets consisted of three LP diets containing 1.5% less crude protein (CP) based on the control diets 23 (starter), 21 (grower) and 19% (finishers) of the control than diets. To meet the standardised ileal digestible (SID) AA needs of broilers, the LP diets were supplemented with crystalline essential amino acids (AA). The AA-supplemented LP treatments did not affect the performance parameters. Mansilla *et al.* (2022) tested the effect of feeding energy-reduced diets with a constant digestible lysine (dLys): apparent metabolizable energy (AME) or with increasing Lys: AME on broiler performance. They observed a linear decrease in body weight (BW) with progressive reduction in dietary energy at constant Lys: AME, however, BW exhibited a quadratic response with the incremental changes in the ratio. Although feed conversion ratio (FCR) was statistically lower overall with energy reduction compared to the control, it improved with increasing Lys: ME ratios. They concluded that gradually lowering energy density while adjusting the Lys: ME ratio during the last phases (i.e. 20-42 days) of the production cycle could be a feasible approach to managing feed costs.

The overall aim of the study was therefore to determine the effect diets varying in AMEn and dLys level on growth performance of Cobb 500 broilers. The objectives of the study were to assess the growth performance of broilers fed diets with a constant dLys: AMEn ratio, and variable AMEn, in the period from 14 to 35 days of age (Experiment 1); and to evaluate the growth performance of broilers fed diets with a constant AMEn (12.1MJ/kg) and varying dLys level, in the period from 14 to 35 days of age (Experiment 2).

Chapter 2

Literature review[1]

2.1 Introduction

An in-depth understanding of the association between diet composition and performance becomes increasingly essential for the broiler industry as global demand for premium poultry products keeps rising (Erdaw & Beyene, 2022). Broiler diets provide the bird with energy and nutrients, such as protein, lipids, vitamins, minerals, and feed additives. Energy and protein are the two most critical components of poultry diets, and various studies have studied the influence of these diet components on broiler performance. Energy is a by-product of oxidative phosphorylation of energy-yielding dietary nutrients such as carbohydrates and lipid (NRC, 1994; Wilson, 2017). The bird uses energy to sustain metabolic activities such as digestion and absorption of nutrients, growth (protein accretion), and thermoregulation (Toyomizu *et al*., 2011; Wilson, 2017). Protein is primarily associated with important functions including biosynthesis of skeletal muscle tissue and structural proteins (i.e. elastin), cell signalling (hormones), and acting as enzymes to catalyse essential metabolic reaction (Qaid & Al- Garadi, 2021; Wu *et al*., 2014a). Broiler energy and AA requirements may vary according to factors such as age, strain, environmental changes and production goals, highlighting the need for precise diet formulation (Panisson *et al*., 2022).

This chapter discusses the influence of dietary energy and protein on broiler production. Before exploring the multifaceted relationship between dietary energy and protein, and broiler performance, it is important to understand the context within which these discussions unfold. Over the years, the poultry industry has experienced significant growth and transformation; however, the search for effective and sustainable broiler production practices has been dynamic. Thus, the first section briefly summarises the successes and challenges (feed-related) facing the South African broiler sector. Aspects relating dietary energy and AA, and their effect on broiler performance are discussed in the subsequent sections.

2.2 The South African broiler industry

The broiler industry is an integral part of the South African economy for several reasons, namely, job creation, economic growth, and food security. According to the Department of Agriculture, Land Reform, and Rural Development, the local broiler industry has the largest production value in the

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[1] An article (titled: Effect of varying levels of dietary energy and protein on broiler performance: a review) based on select sections of this review has been published in the World's Poultry Science Journal. https://protect-za.mimecast.com/s/PO-PC76JVlCpRq8XsBeVK8?domain=doi.org

agricultural sector (DALRRD, 2021). Broiler meat accounts for up to 90% of the total poultry meat supply. One of the most critical drivers of the poultry industry's success is the production of an affordable protein source, when compared to other animal-based protein sources, which is accompanied by the largest per capita consumption of all animal protein in South Africa (SAPA, 2021). Despite the positive image, the national and international broiler sectors face multiple challenges, of which high production costs is the main challenge (Chatterjee & Rajkumar, 2015). The annual production price of poultry meat has increased over the past decade, with producer prices currently ranging between R30 and R35/kg (NAMC, 2023; Figure 2.1). Broiler feed prices for all production phases increased from an average of just below R5500/tonne in early 2018 to just below R8500/tonne between January and July 2022 (SAPA, 2022). This trend can be attributed primarily to the increase in the price of raw materials such as maize and soybean (Ravindran, 2013). The ratios of broiler selling price to common energy and protein feed ingredients exhibited a decreasing trend between 2018 and 2022 (SAPA, 2022; Figure 2.2).

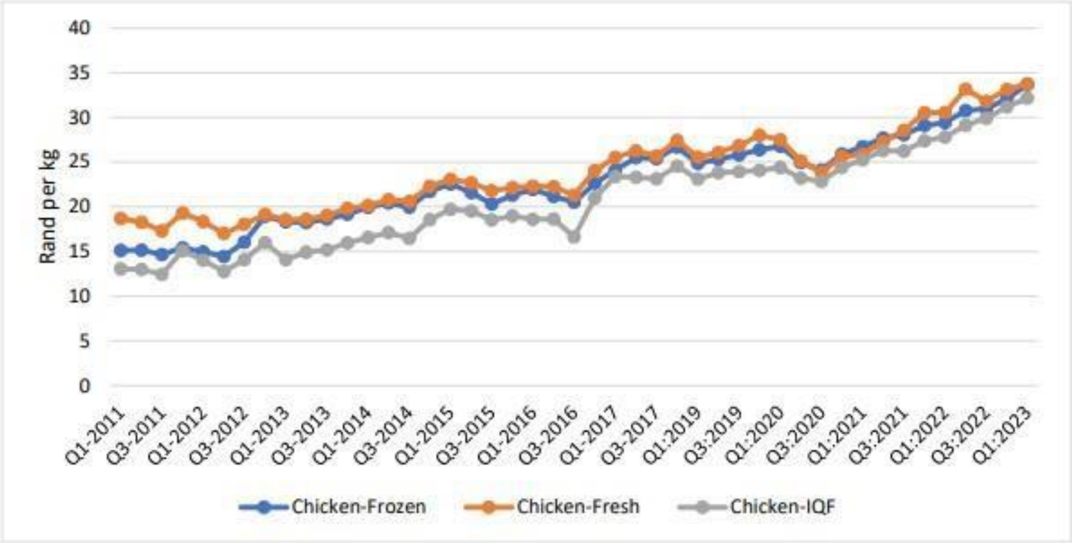

Figure 2. 1 The average broiler producer price per kg from the first quarter of 2011 to the first quarter of 2023, according to the National Agricultural Marketing Council (NAMC, 2023).

The negative impact of cheap poultry meat imports on the local broiler industry has been noted (Jacobsz, 2018). While local producers are battling the constant rise in feed prices as illustrated in Figure 2.2, either due to factors such as drought affecting grain production or increased demand for grains (i.e. for human consumption), cheap imports further complicate matters. Higher feed prices negatively impact profit margins, making it harder for local producers to compete, particularly if they cannot pass on the increased costs to consumers due to price sensitivity (Bagopi *et al*.,2014). Thus, some producers may be forced to scale down or shutdown operations. Nkgadima (2022) indicated that local broiler production increased by about 4% between April 2010 and June 2022; increased import tariffs brought about this change. Therefore, government intervention is crucial in ensuring the local broiler industry is and remain viable and competitive.

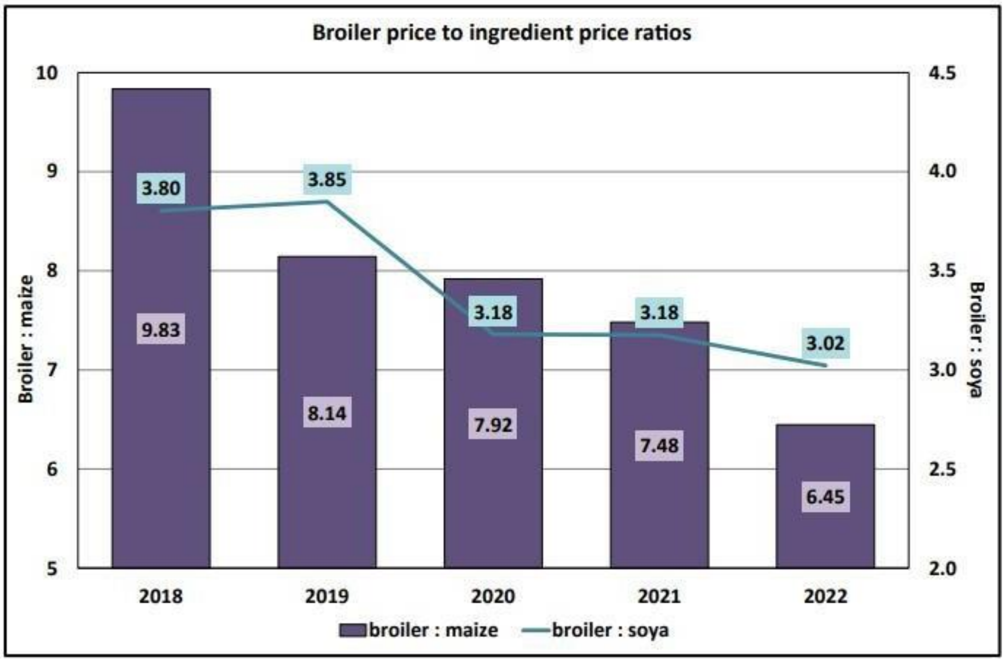

Figure 2.2 The broiler price to ingredient (maize and soybean) price ratios from 2018 to 2022 as reported by the South African Poultry Association (SAPA, 2022).

2.3 The digestive tract and digestive physiology

The avian digestive system comprises the beak, the oesophagus, the proventriculus, the ventriculus (gizzard), small intestine, the large intestine, and the cloaca; as well as various related organs such as the liver (Biesek et al., 2022). Some anatomical and functional differences in the gastrointestinal tract (GIT) of poultry compared to other livestock have been noted. For example, poultry consume a larger proportion of feed in comparison to their metabolic size, and their GIT transit time is around half that of mammals (Moran Jr & Bedford, 2022). The maturity of the digestive tract needs to be considered when formulating diets. The first week of a broiler's life is critical as the GIT is immature. During that week, exponential changes will take place as the bird transitions from utilising nutrients in the yolk to those present in the feed (Ravindran and Adbollahi, 2021). As the birds get older, the GIT matures at a rapid pace allowing for an increased digestibility of nutrients (Khalil et al. 2021). Productive parameters of broilers subjected to different AMEn concentrations at a constant dLys: AMEn ratio from 14 to 35 days of age.

The chemical composition of a complete feed, which consists of crude fibre (CF), crude protein (CP), N- free extract (NFE), and anti-nutritional factors (i.e., phytate), may significantly impact on the nutritional value of a diet, and the effectiveness of energy and protein utilisation (Selle *et al.*, 2000; Jiménez-Moreno *et al.*, 2009; Woyengo & Nyachoti, 2013). Birds lack digestive enzymes to break down substances that are considered anti-nutritional factors, which may include phytate, β-glucans, etc. Studies show that exogenous enzymes such as phytase can mitigate the effect of anti-nutritional factors and improve nutrient use (McCafferty *et al.*, 2022). Stefanello *et al.* (2016) found that AMEn in a maize-soybean-based diet was significantly enhanced by a minimum inclusion of 100 FXU/kg xylanase. Interactions between feed molecules such as AA and non-protein nitrogen (i.e. arginine analogues) can affect nutrient utilisation by broiler chickens (D'Mello, 2003). Musigwa *et al.* (2021) established that multi-carbohydrase enzyme use, significantly improved dietary energy utilisation and enhanced the efficiency of nitrogen use in birds fed a diet containing non-starch polysaccharides (NSPs) with low solubility.

2.4 Dietary energy for broilers

While not disregarding other energy-yielding organic matter such as protein as illustrated in Figure 2.3, poultry diets contain carbohydrates and fats/oils as primary energy sources (Thirumalaisamy *et al.*, 2016; Barzegar *et al.*, 2020). Despite the high energy content in fats, Despite the high energy content contained in fats and oils, carbohydrates constitute a more significant proportion because fat inclusion must be limited due to factors that dictate its utilisation, such as the birds' physiology and its effect on feed quality (Ravindran *et al.*, 2016; Zaefarian *et al.*, 2019). The breakdown of the usually complex sugars in the GIT is dependent on the bioactivity of enzymes such as salivary amylase and pancreatic alpha-amylase (Scanes, 2022). The hydrolysis of starch begins when amylase breaks it into smaller water-soluble molecules, and the other enzymes including maltase and isomaltase further process these into glucose molecules (Carré, 2004; Scanes, 2022). The resulting glucose molecules are absorbed into the enterocytes (small intestinal wall) via sodium-glucose transporters (SGLTs) and enter the portal system for distribution to different organs, including the liver (Selle & Liu, 2019). It is generally believed that glucose absorption principally takes place in the duodenum and jejunum segments of the small intestine. However, Riesenfeld *et al.* (1980) observed a similarity in apparent permeability for glucose between the jejunum and ileum and suggested that the differences in absorption are due to the differences in glucose input or flow into the segments as the anatomy dictates. In the hepatocytes, glucose is broken down into precursor molecules to generate energy for use in maintenance and production or stored as an energy reserve in the form of glycogen (Li *et al.*, 2019).

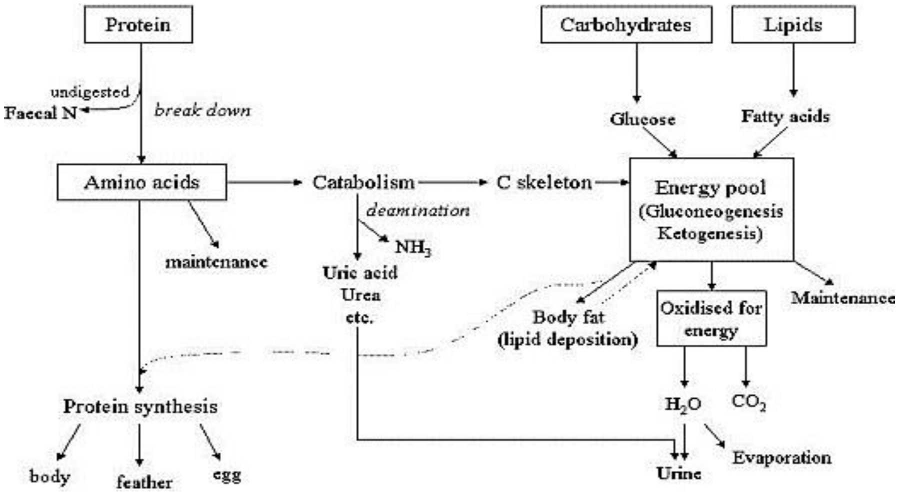

Figure 2. 3 Metabolism of protein and energy-yielding substrates in broiler diets (Kim, 2014).

Ingested dietary fats undergo emulsification and digestion (hydrolysis) in the small intestine. Upon mixing with bile salts, lipids are emulsified into tiny droplets (micelles), and pancreatic lipase completes the digestion (Alvarenga *et al.*, 2011). The droplets are hydrolysed into fatty acids (FA) and monoglycerides, which get absorbed by diffusion into the mucosal cells where portomicrons are synthesised for transport to the hepatocytes (Alvarenga *et al.*, 2011; Zaefarian *et al.*, 2019). Rodriguez-Sanchez *et al.* (2019) found that the duodenum is the focal point for the digestion of fat, while the jejunum is the main site where fat absorption takes place. They discussed that the ileum plays a crucial role in the absorption of FA. Hence, the ileum was responsible for the increased fat utilisation seen in birds fed a diet containing unsaturated fats compared to the group fed saturated fats at 14 days and improved utilisation of the latter diet up to 35 days. The major differences were observed in the last two segments of the small intestine; the authors suggested that the assimilation of FAs is notably extra limiting compared to hydrolysis. An early study by Krogdahl (1985) discussed that the quality and quantity of dietary lipids influence the secretion of bile salts crucial for emulsification and micelle formation. Important factors that influence the energy content and contribution of fats in poultry diets, include the degree of lipid saturation, chain length, free fatty acids, age of the birds, and inclusion level (Ravindran *et al.*, 2016).

Broiler age and genetic makeup affect the bird's capacity to retain feed energy, and some genetic strains tend to retain more fat in the form of an abdominal fat pad, than others (Jorgensen *et al.*, 1990; Caldas *et al.*, 2019). According to Lopez and Leeson (2008), energy consumed by the bird is typically allocated into energy retained in body tissue and heat production. Heat production in thermoneutral conditions signifies heat related to the utilisation of energy for maintenance and production (growth), which accounts for about 52–64% of metabolizable energy (ME) intake in young broilers. The majority of the total energy retention in the chicken's body comprises energy stored as fat (adipose tissue) as well as protein (primarily muscle), and in adult birds, the efficiency of utilisation of ME above maintenance requirements range from 70 to 84% for fat deposition, while in growing birds, it varies from 37 to 85% (Lopez & Leeson, 2008). In essence, the energy that is not utilised for maintenance or production, will be stored primarily as fat, reducing the efficiency of energy utilisation for skeletal muscle growth. Thus, affecting overall carcass composition, particularly during the late stages of production (Musigwa *et al.*, 2020).

2.4.1 Categorization of dietary energy

Chickens must take in enough feed to meet their energy needs to sustain metabolic processes in the body, maintenance, and production (growth). Digestible energy (DE) cannot be measured under non-surgical trial conditions because in poultry, unlike in ruminants and swine, urine and faeces are excreted collectively through the cloaca (Pirgozliev & Rose, 1999; Barzegar *et al.*, 2020)**.** Wu *et al.* (2020) pointed out that attempts to measure DE using surgical techniques can be made, but the digestion of surgically modified chickens might differ from normal birds; therefore, DE as an estimate lacks feasibility in this regard.

Metabolizable energy (ME) is the most accepted and extensively utilised approach for describing the energy density of feed ingredients and complete diets; energy requirements are usually stated using the same units (Swick *et al.*, 2014; Barzegar *et al.*, 2020). It is defined as the feed energy available for use by the bird derived from the difference between feed gross energy (GE) and the energy of the excrement (a mixture of faeces and urine for poultry) (NRC, 1994; Wu *et al.*, 2020). At present, apparent and true metabolizable energy (considering endogenous energy lost in excreta) (AME, TME) are the two important estimates in practical terms, and each of these can be corrected to zero nitrogen (N) retention to get AMEn and TMEn (Yaghobfar, 2016). Uric acid produced from the oxidation of protein retained as body tissue has a GE value per given weight of N (about 0.034MJ/g), which is the basis of the theory for zero nitrogen correction in broiler diets (Lopez & Leeson, 2007; Abdollahi *et al.*, 2021).

Wolynetz and Sibbald (1984) evaluated the association between AME and TME equations and N correction effect on each derivation. According to the authors, both AME and AMEn significantly underestimate bio-available energy (BE) of feed ingredients at low feed intakes (FI), and TME tends to overestimate BE at the same levels of FI, but the difference is less than the underestimation of the former estimates; TMEn provides an acceptable estimate for all ranges of FI. Within the practical (unlimited) feed consumption range, AME is less than AMEn when retained nitrogen (RN)<0, while AME is greater than AMEn when RN>0. The accuracy of both TME and TMEn is lesser at a specific FI level than their corresponding alternative estimates. This difference can be attributed to the variance related to the excreta energy of the starved control group. They concluded that theoretical considerations and experimental data point that TMEn is the most accurate estimate of BE.

Lopez and Leeson (2007) re-evaluated the relevance of N correction for the assessment of ME and concluded that since there was less variability in AME_n values compared to when expressed as AME, it appeared there were sufficient differences in growth amongst birds to justify applying the adjustment factor in broiler diets. However, Abdollahi *et al.* (2021) argued that this correction for modern broiler chickens imposes a penalty on the energy value of primary protein sources; this retribution is even worse for feedstuff with superior-quality protein. Therefore, they propose that poultry feed should be formulated based on uncorrected AME values, mentioning that this adjustment bears potential cost-reducing benefits and merits further investigation.

At present, AMEn is the default estimate; for TME, it has been proven that fasting of control birds overestimates endogenous energy loss, among other limitations, such as the use of adult male birds, while it has been demonstrated that age affects ME values (Wu *et al.*, 2020). Trial results obtained by Yang *et al.* (2020) indicated that ME measurements determined earlier than 14 days of age could result in lower absolute values than those determined in older birds; there was a linear increase for AME and AMEn between 7 to 28 days. They also noticed that ME values determined on older birds might overestimate BE of commercial starter diets. Early work by Zelenka (1997) found a significant effect of broiler chickens' age on AMEn values; they increased with age for the diet with a narrower ME to protein ratio; while they observed a parabolic trend for the diet with a wider ratio with values increasing to a limit at 37 days of age. Under *ad libitum* feeding conditions, hens fed the diet with higher energy: protein ratio reported significantly decreased AMEn values when FI increased. There is limited data on the impact of gender on feed ME values. Ravindran *et al.* (2004) found that AMEn values computed at week 3 were unaffected by the sex of the broilers; however, they were significantly higher for male broilers compared to those for females at 6 weeks. of age. Bird sex has been suggested to affect the digestive capacity of fowl because of the differences in endogenous energy loss, gut morphology and function, and metabolic activity of gut microbes (Wu *et al.*, 2020). Based on the information from these publications, sex at specific age intervals could be a factor to be considered in future ME bioassays.

Studies indicate that net energy (NE), determined by subtracting heat energy lost through metabolic activity from the AME value) is the closest approximation of the actual feed energy value, and its use instead of the ME system may benefit least cost formulation for broiler diets (Pirgozliev & Rose, 1999; Noblet *et al.*, 2010; Swick *et al.*, 2014; Yaghobfar, 2016; Noblet *et al.*, 2021). Still, some publications argue that its direct measurement is complicated in practice. Zuidhof (2019) proposed that the ease of measuring ME and the complex nature of determining NE (i.e., resolving heat production sources into their basic contributory effects) imply that the latter offers no added benefit over the current default system for poultry feed formulation. Van der Klis and Jansman (2019) also concluded that its additional value over the current is yet to be proven to warrant its use in the broiler feed industry. Nonetheless, preliminary work has demonstrated the possibility of predicting with accuracy NE, HI and the efficiency of energy utilisation (DE: ME) using AME values as well as feed chemical composition (Swick *et al.*, 2013; Carré *et al.*, 2014; Wu *et al.*, 2019). In the current work, ME is used as a general reference to feed energy.

2.4.2 *Setting dietary energy levels*

Energy density is typically considered as a starting point when formulating poultry feeds; thus, selecting a suitable concentration will, under favourable conditions, result in an improved bird performance at cost-effective diet prices (NRC, 1994; Martins *et al.*, 2016). The rationale behind this idea stems from the commonly accepted fact that chickens tend to consume enough feed to satisfy their energy requirements (constant ME intake) for specific physiological processes associated with maintenance and production (Ferket & Gernat, 2006). Numerous studies support this argument as the basis for physiological control of FI in domestic fowl, amongst other factors such as gut capacity, bird strain and form of feed (Richards & Proszkowiec-Weglarz, 2007; Ahiwe *et al.*, 2018; Massuquetto *et al.*, 2020; Maharjan *et al.*, 2021). Conversely, some research suggests that continuous genomic selection for better-performing birds has, over time, resulted in broilers that are less responsive to dietary energy density and will eat to their death (Classen, 2017; Ahiwe *et al.*, 2018). It is, therefore, crucial to reflect on the balance between available energy and other essential nutrients, such as protein and calcium, to avoid deficiencies or toxicities from overconsumption by the birds (Massuquetto *et al.*, 2020). Table 2.1 shows ME and NE models that may be used, with coefficients derived through research to determine energy requirements for broilers and subsequently to specify the required energy density of diets.

Table 2. 1 Equations (models) for predicting energy requirements for broiler chickens per day in a production cycle.

System	**Energy requirements model**	**Reference**
Metabolizable	$ME = W^{0.75}(307.87 - 15.63T + 0.3105T^2) + 13.52Fg + 12.59Pg$	(Sakomura, 2004)
Net	$NE = W^{0.75}(212.83 - 9.658T + 0.188T^2) + 9.37Fg + 5.66Pg$	(Sakomura *et al.*, 2005)

$W^{0.75}$: birds metabolic body weight (kg); **T**: temperature (^{0}C); **Fg**: fat gain (g/d); **Pg**: protein gain (g/d). Energy values were expressed in Kcal/bird/day.

Early National Research Council (NRC, 1994) publications recommended about 13. 39 MJ/kg ME for broilers for all production phases; however, several works have focused on modelling responses for dietary energy to allow for a broader and flexible range of choices on feedstuff and determining optimal energy densities for bird- related factors (i.e. strain) and the different phases (Classen, 2013). Currently, most diets contain energy levels below the NRC value in the early stage of growth, some even to finisher; energy levels go up with age in response to the increase in energy demands associated with body weight (metabolic) gain (Arabi, 2015). Modern broilers do not respond well to extreme temperatures, and the effect on production performance is well documented (Olanrewaju *et al.*, 2010; Purswell *et al.*, 2012). Hence, marked alterations in dietary energy specifications may be observed under different ambient temperatures governed by the non-linear effect (Table 2.1) temperature has on maintenance (thermoregulation) requirements. Hot ambient temperatures raise the most concern among broiler producers. Some researchers recommend a reduction in ME levels proportional to CP for certain bird strains (Sa'adu *et al.,* 2018); while others suggest moderate increases in ME with the inclusion of supplements such as good-quality fat to help mitigate the heat stress (Ghazalah *et al.*, 2008; Infante-Rodríguez *et al.,* 2016).

2.5 Dietary protein for broilers

For all livestock, ruminants, and non-ruminants, including poultry, protein is an essential feed component, and multiple studies acknowledge its crucial role in growth and meat quality. It is usually expressed as crude protein (CP), which could be a percentage (%) or g/kg, typically on a dry matter (DM) basis (Åkerlind *et al.*, 2011). Generally, the CP content of feedstuff and complete feeds is determined by applying the standard conversion factor nitrogen (N) x 6.25 (Åkerlind *et al.*, 2011). However, this approach is not without criticism, the concern mainly being that it does not account for the variation in N: protein ratios observed for different feed ingredients and the possible contribution to N output in assays by other N-bearing non-protein N (NPN) compounds that may be contained in feedstuff (Sriperm *et al.,* 2011).

Protein (constituent amino acids) is a major and essential component of broiler diets, and data show that its intrinsic properties such as the presence of trypsin inhibitor in soybeans can impact on the digestion and amino acid (AA) bioavailability (Leske & Coon, 1999; Gilani *et al.*, 2012; Macelline *et al.,* 2021). The ingested protein undergoes partial digestion in the proventriculus, where the digesta is mixed with gastric juices. Gastric HCl supports protein digestion through the conversion of inactive gastric proteases (zymogens), such as pepsinogens A and pro-chymosin, to active proteases, such as pepsin and chymosin (Chen, 2017). It also aids the process by denaturing (unfolding) dietary protein to allow the breakdown of peptide bonds by proteases (He *et al.*, 2021). Pancreatic proteases, including trypsin, chymotrypsin, elastase, and carboxypeptidase (A and B), further break down feed protein in the small intestine after it has been partially digested in the proventriculus (Coon, 2002). Prior to being absorbed by enterocytes in the small intestinal mucosa, CP must first be dissolved into di- and tripeptides or free AA in the gastro-intestinal tract (GIT) (He *et al.*, 2021). Enterocytes absorb free AA mainly in two paths, first through a facilitated sodium (Na^+) independent path for basic AA as well as the smaller and larger neutral AAs and via active transportation (Na^+ gated channels) for free acidic AA as well neutral AA of different proportions (He *et al.*, 2021). The apical-membrane Na^+- independent, H^+-driven peptide transporter 1 permits the absorption of di- and tripeptides by the enterocytes; they then undergo rapid hydrolysis within the cells by cytosolic peptidases to form free AA (He *et al.*, 2021). Absorbed-free AA enter the portal system for distribution (systemic circulation) to different organs (Zaefarian *et al.*, 2019). Amino acids available to the chicken can be used for various purposes, including muscle tissue synthesis and excess AA are catabolised in the liver, and uric acid is formed in the process (Stevens, 1996; Zaefarian *et al.*, 2019).

2.5.1 *Amino acids*

Feed protein consists of various (AA), some of which the birds are not able to metabolically synthesise within their bodies and need to be supplied through diet (essential AA), and ones that they can produce (non-essential AA), generally assumed not be required in the diet (D'Mello, 2003). However, Wu (2014b) suggests that this group of AA is not getting enough attention yet also plays a crucial role, such as promoting gut development, functionality, and integrity (i.e. aspartic and glutamic acid). The basis of the concern could be that there are possibilities that modern broilers may not be producing some of these AA in enough quantities to ignore their inclusion in diets. Feedstuffs used in poultry diets vary in CP content and AA profile (concentrations), and differences in plant varieties (through genetic breeding, biofortification), processing during feed manufacturing and the sources of the ingredients may affect their nutritional (protein) value as shown in Table 2.2.

Table 2. 2 Crude protein and essential amino acids (AA) profile (concentration) of different feedstuffs used in broiler diets, data obtained from three independent studies.

	(Panda *et al.*, 2014)[1]		(Leske & Coon, 1999)[2]		(Kim *et al.*, 2012)[3]	
	NM	**QPM**	**CSBM**	**EESBM**	**FM**	**MBM**
CP (%)	8.94	9.71	48.6	66.5	63.25	54.31
EAA (%)						
Lysine	0.254	0.392	3.41	4.74	4.98	2.93
Arginine	0.394	0.632	3.71	4.89	3.82	3.67
Methionine	0.172	0.183	0.77	1.07	1.8	0.75
Met+cysteine	0.353	0.454	-	-	-	-
Isoleucine	0.281	0.307	2.36	3.13	2.66	1.52
Threonine	0.296	0.364	1.98	2.67	2.49	1.61
Tryptophan	0.062	0.082	-	-	1.95	0.36
Valine	0.394	0.514	2.28	3.19	4.03	2.29
Histidine	0.242	0.373	-	-	1.45	1.03
Leucine	1.041	0.876	3.74	4.9	4.61	3.27
Phenylalanine	0.423	0.417	-	-	2.43	1.75

EAA essential amino acids; **NM** normal maize; **QPM** quality protein maize; **CSBM** Control Soybean meal; **EESBM** ethanol-extracted Soybean meal; **FM** fish meal; **MBM** meat & bone meal. **1**) Values standardised to 880 (g/kg) dry matter (DM); at ≥ 50% substitution rate, QPM resulted in a significant improvement of production parameters and carcass qualities. **2**) Values expressed in DM basis (44% CP Soybean meal); ethanol extraction improved DM and average AA digestibilities by 63.3 & 91.6% compared to 52.1 & 88.0% for NSBM, respectively.**3**) Values expressed on an as-fed basis; ingredients sourced commercially, available to broiler producers

Amino acids (AA) in poultry feed are usually expressed on an ideal AA profile basis, where all essential AA are included at different ratios relative to 100% Lys as the limiting AA (Bao, 2020). Lysine is regarded as the second most limiting AA in standard maize-soybean meal rations after methionine (Met); and its close association with body protein synthesis makes it a reliable reference for a balanced AA profile (Siqueira *et al.*, 2013). Diets may contain bound AA obtained primarily from protein sources such as soybean meal and, as currently is standard practice, the rapidly absorbable non-bound AA (i.e. crystalline and synthetic AA) (Selle *et al.*, 2022). Van Harn *et al.* (2019) found that reducing CP (by partially replacing Soybean meal) by 2.2 to 2.3% and adequate supplementation of essential AAs did not impair bird performance, while it resulted in better litter quality and a lower incidence of footpad dermatitis. Selle *et al.* (2022) argue that bound and non-bound AA are not biochemically equivalent, particularly concerning the dynamics of their digestion, absorption, and metabolic fate (utilisation by the bird). They put forth that researchers are probably overlooking this distinction, a mistake that could be hindering progress in the development and reception of lower-CP diets in the broiler feed industry.

Growth potential is genetically determined; as a result, AA requirements for the different types (i.e. broiler or layer), breeds, and strains vary (Ravindran, 2012). Protein requirements for poultry essentially signify the bird's N requirements supplied through AA intake (Waldroup *et al.*, 2005). Models which can be used to determine these requirements include those shown in Table 2.3, amongst others**;** for in-depth parameter description and determination, see the original publications as referenced. Some research work has established that the differences (also considering the effect of age and gender) may be due to variations in the effectiveness of digestion, assimilation and metabolic fate of the acquired nutrients (Doeschate *et al.*, 1993). Han and Baker (1993) tested the effect of bird-related factors and temperature on the Lys requirements of broiler chickens. They found that male broilers required higher levels of digestible Lys than females for maximum BWG and FCR, while heat stress significantly decreased both parameters by up to 22% for both sexes, possibly due to a multifactorial impairment of FI and nutrient utilisation. They also categorised chicks by initial BW and found that both groups required a similar amount of AA to reach maximum growth potential (BW). In contrast, the heavier group required more Lys to achieve maximum FCR.

Table 2. 3 Representative models that can be used to estimate or determine amino acid (AA) requirements for broiler chickens.

Model type	**Equation**	**Reference**
Applied N-use[1]	$ILAA = \frac{(lnNRmaxT - \ln(NRmaxT - NR))}{(16qc^{-1})}$	(Liebert, 2008)
Factorial[2]	$dAAI = [(AAm \times BPm^{0.73} \times u) + (FL \times FP \times AAf)] + \frac{(AAb \times BPD + AAf \times FPD)}{k}$	(Sakomura *et al.*, 2015)

1: ILAA, Intake of limiting AA (LAA) (mg/BWkg$^{0.67}$/d); **InNRmaxT**, theoretical maximum N-retention (NR); **bc^{-1}**, the gradient between the dietary concentration of LAA (c) (g/100g CP) & protein quality of the feed (q). **2: dAAI**, digestible AA intake (mg/bird) per day; **AAm** requirements (mg/d) for maintenance (BPm0.73*u) where BP is body protein and u is the birds level of maturity; **FL**, loss of feathers equivalent to 0.01(g/g/d); **FP**, feather protein (g); **AAb** AA required for featherless body tissue growth (mg/d); **AAf**, AA required for feather growth (mg/d); **BPD** rate at which protein is deposited in featherless carcass (g) and feathers (**FPD**) (g/d); **k**, the efficiency of AA utilisation for BPD and FPD

Therefore, optimising protein supply for broiler chickens requires a good understanding of their physiological needs for a particular production parameter or goal and adjusting them properly for cost-effective feeding (Dozier *et al.*, 2008). The NRC (1994) recommended a range between 20 and 23 CP% and specified requirements for various AA; however, current research papers point out that adjustments are necessary, which might continue to be the case from here on, considering the fast rate of genetic improvement (Dozier *et al.*, 2008). Studies may recommend levels above or sometimes below NRC values based on different models for optimal performance or a specific production parameter (Table 2.4).

Table 2. 4 Recommendations of select essential amino acids (AA) as per National Research Council (NRC) (1994) compared to research work of the recent years.

				Recommendation (%)		
EAA	**Age (d)**	**PP**	**CP (%)**	**dAA**	**Diff**	Reference
Lys	0- 21	-	-	1.10	-	(NRC, 1994)
	21- 42	-	-	1.00	-	
	1-8	FCR	-	1.36	123.7	(Siqueira *et al.*, 2013)
	8-21	FCR	20	1.19	107.9	
	22-35	MP		1.16	116.0	(Bernal *et al.*, 2014)
Arg	0- 21	-	-	1.25	-	(NRC, 1994)
	21-42	-	-	1.10	-	
	0- 21	MP	23	1.50	120.0	(Khajali *et al.*, 2018)
	21-42	BY	20	1.26	114.5	(Basoo *et al.*, 2012)
Met	0- 21	-	-	0.50	-	(NRC, 1994)
	1-10	ADG	22.8	0.38	76.0	(Lee *et al.*, 2020b)

EAA, essential AA; **PP**; production parameter; Crude protein (CP) for NRC at 23% & 20% for respective periods; **dAA** digestible amino acid; **Diff** percentage difference from the NRC value for corresponding age period; **FCR**, Feed conversion ratio; **MP**, maximum performance post starter phase; **BY**, breast yield; **ADG**, average daily gain

2.5.2 *Amino acid digestibility*

It is widely acknowledged that AA digestibility is a crucial indicator of bioavailability; therefore, proper evaluation of feedstuff is vital if the birds are to be fed an AA-balanced ration. It may differ depending on several factors, including bird-related such as age, sex, and strain, as well as feed- related factors such as the quality of dietary ingredients and feed additives (i.e. enzymes) (Barua *et al.*, 2021; Fortes *et al.*, 2022). Parsons (2020) concluded that since AA digestion is less effective in younger birds, higher inclusion levels of good-quality protein sources or individual AA during the early weeks of production may result in better performance at finishing. Based on the level of AA intake and NR (AA accumulation in the body) for birds at 2 and 6 weeks. it is estimated that the effectiveness of digestible AA utilisation for growth is 65.5% and 60.3%, respectively; and the values for the different AA may vary from 40% to 79% depending on growth phase (age) and feed properties (He *et al.*, 2021).

Generally, there are two approaches to determining AA digestibility, the total tract (excreta) and ileal AA digestibility. Some researchers propose that the latter gives more accurate values due to the possible AA alterations by microorganisms found in the lower gut (caeca) skewing AA digestibility values, hence the most preferred for the formulation of poultry diets over its alternative (Dublecz *et al.*, 2006; An *et al.*, 2020). It has also been determined that there are significant differences among the same (i.e. maize varieties) and different feed ingredients (i.e. wheat vs soybean) commonly used in poultry diets regarding ileal and total protein digestibility (Parsons, 2020). The ileal digestibility of AA can be subdivided into apparent and standardised ileal digestibility (AID and SID, respectively). The difference between the two procedures is that SID is adjusted for basal endogenic AA losses, and some research works favour its use in broiler diet formulation over AID, claiming the latter estimate may devalue (underestimate) some feedstuffs (Lemme *et al.*, 2004; Cowieson *et al.*, 2019). Adedokun *et al.* (2009) found that AID and SID values obtained from birds that had their caecum surgically removed presented some similarities for select feedstuff but not all. The ingredients in this study ranged from plant-based (i.e., maize and soybean) to those of animal origin (i.e. meat and bone meal), suggesting the difference is beyond just the likely effect hindgut microbes might have on the estimates.

Nutrient additivity in complete diets is central for improved precision in the formulation of poultry feed (Kong & Adeola, 2013; Cowieson *et al.*, 2019). And it has been established that excreta-determined (total) AA digestibility values are less additive than ileal values, probably because they are utilised to a lesser extent by the birds compared to the latter (An *et al.*, 2020). (An *et al.*, 2020) indicated that AID may underestimate AA digestibility of poor quality or low protein (AA) ingredients and AID may display additivity for some AA, while SID was additive regardless of AA identity. Table 2.5 shows that maize had the most significant percentage differences between the two approaches for Lys digestibility, indicative of possible underestimation of AA digestibility. The differences in Lys digestibility for maize across the studies may be due to the differences in protein biological value possibly influenced by crop variety (i.e. normal vs. quality protein maize).

Table 2. 5 Digestibility coefficients of lysine (Lys) in primary feed ingredients as a single major nutrient source or mixed as is the case in standard maize-soy based (complete) broiler diets.

Parameter	(An *et al.*, 2020)[1]			(Cowieson *et al.*, 2019)[1]			(Kong & Adeola, 2013)[2]		
	M	SBM	Mix	M	SBM	Mix	M	SBM	Mix
CP%	7.10	20.00	20.00	7.00	19.00	20.00	7.15	20.23	20.35
				Lys digestibility coefficients					
AID	0.821	0.934	0.933	0.564	0.808	0.794	0.738	0.836	0.865
SID	0.889	0.945	0.946	0.699	0.820	0.822	0.873	0.867	0.878
Diff (%)	7.75	1.17	1.38	21.38	1.47	3.47	16.76	3.64	1.49

1: Calculated crude protein (CP) and **2:** Analysed CP**; M** maize; **SBM** soybean meal; **Mix** maize & SBM for additivity; **AID** apparent ileal digestibility; **SID** standardized ileal digestibility

2.5.3 *Effect of amino acid deficiency and imbalances*

Across all classes of production animals, including poultry, nutrient deficiencies and imbalances have been shown to affect production directly by retarding growth retardation, or indirectly by resource wastage (Hurwitz *et al.*, 1998). Research has shown that poor protein quality or a substantial shortage or excess of AA, particularly of essential AA (i.e. Lys and Met) in animal feed, culminates in a decline in the efficiency of protein utilisation, ultimately decreasing productivity (Hafez *et al.*, 1978; An *et al.*, 2020; Gracheva *et al.*, 2020). Ueda *et al.* (1982) found that supplementing Met above the recommended level resulted in a significant reduction in production parameters as well as in energy and protein utilisation. Amino acids absorbed in excess will undergo deamination (catabolism), a process that yields ammonia which, if it accumulates in the bloodstream, will cause toxicity and impair performance (Chen *et al.*, 2020). The bird's adaptation mechanism is to convert it to uric acid for excretion; this process has been associated with excessive water consumption that could lead to poor litter quality, possibly culminating in welfare and health issues (Francesch & Brufau, 2004).

Sigolo *et al.* (2019) found that supplying Lys and Met at 100 to 120% above the breeder's recommendations did not have any added benefits on overall bird performance, so the excess possibly gets excreted. Dietary digestible Lys deficiency during the early growth phase may decrease overall broiler performance and carcass quality measures (Kidd *et al.*, 1998). Besides production performance, nutrition, including AA, also plays a crucial role in promoting bird health, ensuring an efficient production cycle. A Met-deficient diet will impair liver lipoprotein exportation, result in excessive fatty acid accumulation in the hepatocytes, and consequently predispose the birds to liver failure (i.e. fatty liver syndrome) and decreased production (Peng *et al.*, 2018). Chen *et al.* (2003) discussed that a Lys deficient diet could reduce the birds' immune response to Newcastle disease. Excess of a particular AA can affect the bioavailability of others, thereby inducing a deficiency. Supporting this claim, Ospina-(Ospina-Rojas *et al.*, 2020) found that higher leucine levels altered valine and isoleucine requirements (recommendations) for optimal performance of birds fed low-protein rations.

2.6 The effect of dietary energy to protein ratio

Every metabolic process in the chicken's body, including protein synthesis, requires energy, and studies have shown that providing adequate dietary energy will maximise the ration’s protein deposition potential (Kim, 2014; Classen, 2017). Early research by Pesti and Smith (1984) reported a close dependence of the efficiency of feed utilisation and production performance (growth) on both dietary ME and protein and concluded that for an accurate prediction of broiler responses, these major feed properties must be well-determined. Therefore, it is evident that determining a specific protein requirement or recommendation without considering the birds' energy needs for a particular production goal is meaningless (Zaman *et al.*, 2008). Thus, exists a special relationship commonly referred to as the CP:ME (Liu *et al.*, 2016) or Lys: ME ratio (Chen *et al.*, 2019), with Lys regarded as the reference for the ideal AA balance (Rosebrough & McMurtry, 1993; Khoddami *et al.*, 2018). Chen *et al.* (2019) recommended a proportional increase in dietary AA when energy-dense diets are fed to ensure better production performance and improve carcass quality.

Early work by Kita *et al.* (1989) evaluated the influence of dietary energy on whole-body protein turnover (PT) of broiler chickens. They noticed a significant linear increase in the fractional protein synthesis (FSR) and degradation rates (FDR) when feed energy level increased from 12.6 to 15.9 MJ/kg, however, FDR occurred with less sensitivity. Jackson *et al.* (1982) reported that increments above 28% CP or 14.23 MJ/kg ME resulted in diminishing returns, as was also the case with excessive amounts of each nutrient relative to the other. Before returns were reduced, male broilers could tolerate a higher dietary protein level (24%) than their female counterparts (20%). Musigwa *et al.* (2020) evaluated energy dynamics, nitrogen (N) balance, and performance in broiler chickens offered diets containing different CP densities. Their results presented higher efficiency of N utilisation in birds raised on low protein diets (18.9% CP and 0.99% Lys) compared to high protein diets (23.5% CP, 1.2% Lys). Nevertheless, this group also reported increased energy intake in relation to N retained; the excess was deposited as body fat, adversely affecting FCR. Thus it is necessary to consider the physiological and equally critical, the practical consequences of the interactions between energy intake and protein utilisation and vice versa when determining the dietary requirements for each nutrient as well as the optimal balance.

2.6.1 Feed consumption

The first issue for optimising broiler diet responses is achieving the highest daily voluntary FI necessary to achieve the required amount of nutrient intake (Liu *et al.*, 2016); however, the factors that influence it are multifactorial (Forbes, 2013; Classen, 2017). Literature indicates that if FI is too low, a large proportion of ME intake will mainly be partitioned for maintenance requirements; it will also lead to inadequate intake of other essential nutrients, resulting in a decline in production (Forbes, 2007). It is generally assumed that dietary ME density, together with other factors (such as gut capacity), have a regulatory impact on FI, which in turn governs the intake of other nutrients, including protein (Forbes, 2013; Nascimento *et al.*, 2020). Hu *et al.* (2019) reported that high feed energy (14.64 MJ/kg ME) reduced the appetite of broilers and downregulated the signalling of the AMP-activated protein kinase (AMPK) enzyme, which is primarily responsible for promoting the intake of the energy-yielding substrate such as glucose and fatty acids. Conversely, the diet with lower dietary energy (12.13 MJ/kg) had the opposite effect.

Niu *et al.* (2009) found that both dietary ME and protein substantially improved feed efficiency in the early production phase (1-21d), while only ME had a significant effect on FI. Leeson *et al.* (1996) tested the effect of dietary energy and protein alterations by dilution (0 to 50%, 10% increments) in late production (35-49d). This was achieved by including oat hulls and sand at different levels while keeping protein relatively constant in one experiment and in the other the same ingredients were used with graded increments for the diluents to achieve diverse energy and protein ratios. For both investigations, nutrient dilution resulted in a significant increase in FI; however, this affected energy and protein balance, particularly evident in the second experiment where there was a noticeable decrease in production performance. A significant linear decline in the efficiency of feed utilisation, carcass, breast and fat pad yields was observed as the dilution rate increased from 0 to 50% potentially due to reduced dietary CP concentration and significantly low ME intake despite the significant increase in FI. The results also suggested that male broilers at this stage of production are better able to tolerate energy- deficient diets; however, an adjustment in FI of about seven days is necessary for the birds to adapt to the new dietary alterations. Nonetheless, it was also determined that diluting ME and CP by up to 5% did not significantly affect FI or broiler performance (from 1 to 42 days of age) (Azizi *et al.*, 2011).

Abdel-Hafeez *et al.* (2016) evaluated the effect of diets with varying dietary ME and CP levels at normal (58 MJ/kg) ME: CP ratios (as per NRC recommendations, 13.38 MJ/kg, and 23% CP) and wider ratios (60, 61 and 62 MJ/kg protein). They reported an overall decline (between 3 to 3.8%) in FI with increasing ME density across the experimental diets. Better protein intake and utilisation efficiency were observed with proportional energy and protein increments (maintaining the normal ratio) up to the point where inclusion levels were beyond the bird's potential for maximum protein deposition. There are contradictory reports on the effect of ME on FI. In some instances, there are no significant effects, while others report an increase in ME at a constant ME:CP ratio decrease FI, (Table 2.6). The response in FI when altering the ratio could be non-linear (Table 2.6); thus to identify the optimal ratio, the use of modelling such as using non-linear multiple regression is appropriate.

Despite the generalised assumption concerning the impact of dietary ME on FI, results obtained by Liu *et al.* (2019) demonstrated that dietary protein, particularly the density of AA in the diet, may also influence FI. Increasing ME from 11.25 to 13.5 MJ/kg independently from dietary AA density resulted in a decline in FI, while in a range of dLys: ME ratios, different dLys (ranging from 0.92 to 1.21%) levels exhibited a quadratic effect on FI; the treatment diets were fed to male broiler chickens from 21 to 35 days of age. Khwatenge *et al.* (2020) reported varying effects on receptor expression of hormones that influence appetite and satiety in broiler chickens, such as ghrelin, leptin, and adiponectin. Diets comprised different Lys densities (75%, 100% and 125% of the NRC recommended levels). Lower Lys levels upregulated receptor expression, while higher levels had the opposite effect on some of the hormones. Birds that were offered high (125%) Lys showed enhanced growth rates compared to their low Lys counterparts. At the same time, they also exhibited signs of elevated hepatic ghrelin levels, and higher amounts are associated with liver cirrhosis in humans (Tickle *et al.*, 2018; Elaghori *et al.*, 2019). This could signal the beginning of liver damage since excess AA are deaminated by the liver producing toxic by-products such as ammonia before conversion to uric acid for excretion (Zaefarian *et al.*, 2019).

Table 2. 6 Effect of metabolizable energy (ME): crude protein (CP) ratios on cumulative feed intake (FI) of broiler chickens

Strain	**Age (d)**	***T***	**ME (MJ/kg)**	**CP (g/kg)**	**ME: CP (MJ/kg)**	**FI g/bird**	**Diff (%)**	**Reference**
Ross 308	18 - 30	-	12.64	191	66	1364	-	(Hidalgo *et al.*, 2004)
		-	12.82	195	66	1351	-0,96	
		-	13.01	197	66	1349	-1.11	
		-	13.19	200	66	1317	-3.51	
		-	13.38	202	66	1344	-1.48	
		-	13.56	205	66	1348	-1.18	
Ross breed[1]	28 - 56	-	11.30	190	59	2322.7^{a}	-3.45	(Yunana *et al.*, 2019)
		-		210	54	2243.8^{ab}		

		-	12.13	190	64	2260.8^{ab}	2.99	
		-		210	58	2329.5^{a}		
		-	12.97	190	68	2099.9^{b}	8.34	
		-		210	62	2282.7^{a}		
Hubbard	1 - 26	-	11.05	230	55	1922.5^{a}	-	(Kamran *et al.*, 2008)
		-	11.60	220	55	1857.8^{ab}	-3.42	
		-	12.15	210	55	1787.8^{bc}	-7.26	
		-	12.70	200	55	1756.9^{c}	-9.00	
Cobb 500[2]	0 - 14	C	12.70	220	58	401.0^{b}	-	(Akbari *et al.*, 2017)
		1	12.31	220	56	407.5^{b}	1.61	
		2	11.94	220	54	405.5^{b}	1.12	
		3	12.70	220	56	404.2^{b}	0.79	
		4	13.05	220	59	428.5^{a}	6.63	
	14 - 28	C	13.00	190	68	1757.5^{bc}	-	
		1	12.61	184	66	1872.5^{ab}	6.34	
		2	12.22	178	69	1857^{ab}	5.51	
		3	13.00	190	68	1717.7^{c}	-2.29	
		4	13.39	195	69	1906.2^{a}	8.12	

1 strain not specified; **2** Tested dietary ME levels and energy source treatments (T); control (C) based on Cobb recommendations; T1 3% less ME, 3% maise gluten meal (MGM) & 0 Soybean oil (SO); T2 6% less ME, 0% MGM & SO, T3 as recommended, 2.7, 4.2% MGM & 2.5, 2% SO stater and grower respectively; and T4 3% more ME, 4.7, 2.9% MGM, 4.14, 5% SO for the respective phases. Diff difference (%) in FI from the lowest ME value, highest CP same ME or control: values with different letters within a column (for each study) differ significantly ($P<0.05$)

2.6.2 Live performance and carcass composition

The sole purpose of improving broiler genetics is for the realisation of better performance and resource utilisation to improve economic returns, and nutrition plays a crucial role in ensuring these fast-growing birds reach their maximum potential (Tallentire *et al.*, 2016). Supporting this claim is the substantial amount of work done on optimising dietary ME and CP in broiler chicken diets. Holsheimer and Veerkamp (1992) investigated the effect of varying dietary ME and CP levels on broiler performance (Table 2.7). The treatments comprised four diets at low ME density with either normal or high CP (21.0 or 29.7%, ME: CP ratios 57 and 41 MJ/kg of protein respectively) and four diets at a higher ME density 13.38MJ/kg with either normal or high CP (23.1 or 33.6%, ME:CP ratios 58 and 40 MJ/kg protein respectively). The body weight gain (BWG) and FCR were noticeably higher in the high ME diets (2025g and 1.060g feed: g gain), and the 58 ME:CP ratio diet resulted in better carcass yield, while the 41 ME: CP diet yielded significantly high drumstick weights and low abdominal fat together with high CP diets. They concluded that financial implications and production goals would play a key role in determining the best combinations. Liu *et al.* (2016) reported that BWG peaked at a ratio of 17.0 (g/MJ, CP: ME), and FCR reached the lowest level at 17.8 (g/MJ, CP: ME), while nitrogen retention declined as the ratio increased. The authors suggested that the most plausible reason for the reduction in growth rate at the highest CP content could have been insufficient dietary energy.

Table 2. 7 Effect of metabolizable energy (ME), crude protein (CP) and Lysine concentration (Lys) on body weight (BW), body weight gain (BWG), feed conversion ratio (FCR) and abdominal fat of broiler chickens.

Age (d)	ME (MJ/kg)	CP (%)	Lys (g/kg)	BW (g)	BGW (g/bird)	FCR	Abdominal fat (%)	Reference
21–35	11.25		9.2		1111	1.928		(Liu *et al.*, 2019)
	12.375		9.2		1186	1.761		
	12.375		9.2		1117	1.777		
	13.5		9.2		1157	1.648		
	11.25		10.65		1247	1.766		
	11.25		10.65		1144	1.879		
	12.375		10.65		1188	1.736		
	13.5		10.65		1256	1.571		
	13.5		10.65		1125	1.612		
	11.25		12.1		1179	1.750		
	12.375		12.1		1307	1.568		
	12.375		12.1		1211	1.588		
	13.5		12.1		1215	1.497		
0–56	11.7				2111	2.111	6.91	(Holsheimer & Veerkamp, 1992)
	13.38				3143	1.840	6.22	
		23.1			3147	1.966	6.98	
		33.6			2989	1.985	6.16	
			12		2989	1.977	6.65	
			13.6		3146	1.974	6.49	
1–21	12.13			540		1.350	1.19	(Niu *et al.*, 2009)
	12.55			570		1.310	0.34	
	12.97			580		1.270	1.56	
		20		550		1.310	1.41	
		21		560		1.330	1.31	
		22		560		1.290	1.44	
		23		570		1.290	1.29	

42–56	13.54			3626	1203	2.150	1.94	(Dozier *et al.*, 2007)
	13.13			3651	1228	2.220	1.94	Experiment1
		18	9.8	3651	1226	2.130	1.84	
		18	8.3	3625	1205	2.230	2.05	
42–56	13.84			3657	1225	2.197	2.79	(Dozier *et al.*, 2007)
	13.46			3615	1186	2.338	2.61	Experiment 2
		18	9.8	3628	1199	2.234	2.66	
		18	8.3	3644	1211	2.299	2.74	

Niu *et al.* (2009) assessed (from 1 to 21 days of age) different dietary ME (12.13, 12.55 and 12.97 MJ/kg) and CP (20, 21 and 23%), resulting in varying ME: CP ratios (Table 2.7). They found a significant increase in BWG (6.9%) when ME and CP increased, and both ME and CP improved feed conversion efficiency FCE (5.9 and 3%, respectively), while high ME levels (12.97 MJ/kg) significantly increased abdominal fat (23.7%). The authors concluded that a high ME (12.97MJ/kg) and CP between 21-22% provided the optimal ratios. Abdel-Hafeez *et al.* (2016) tested the effect of high feed ME (13.70 to 14.33 MJ/kg) with either standard (23%) or high (23.54, 24.0 and 24.62%) CP levels to give standard (NRC recommendations) and wider ME: CP ratios, respectively, from 1 to 56 days of age. Diets with high ME at standard ratios resulted in faster growth and better FCE than the control, however, the abdominal fat pad was significantly higher than the control groups. There was no significant difference between the standard and wider ratios on breast yield and other organ weights, such as the liver. Also, there was no statistical difference in blood metabolites (health indicators) between the treatment diets, except for liver enzyme ALT when ME was increased. They proposed increasing diet ME (14.02 MJ/kg) and CP levels and 24, 21 and 19% for the respective production phases while maintaining the ME: CP ratio.

Collin *et al.* (2003) discovered that in a range of isocaloric but different CP diets, broilers that were fed low CP (12.5%) partitioned about 57% less ME for protein accretion while they tended to deposit about 33% more as body lipids. Thus, excessive fat accumulation can be associated with the birds' inefficiency in utilising dietary energy for lean deposition, consequently altering carcass composition. Data indicates that proportional increments in dietary ME and CP are fundamental for optimal performance. The increase in ME: CP ratio represents an inverse association between dietary ME and CP densities, where lower ME high CP give narrower ratios and increasing feed ME while decreasing CP content results in wider ratios. In a range of ratios broiler responses will differ depending on specific factor such as the feeding regime or flock management and diet composition as illustrated in Figure 2.5**,** data extracted from Gous *et al.* (2018).

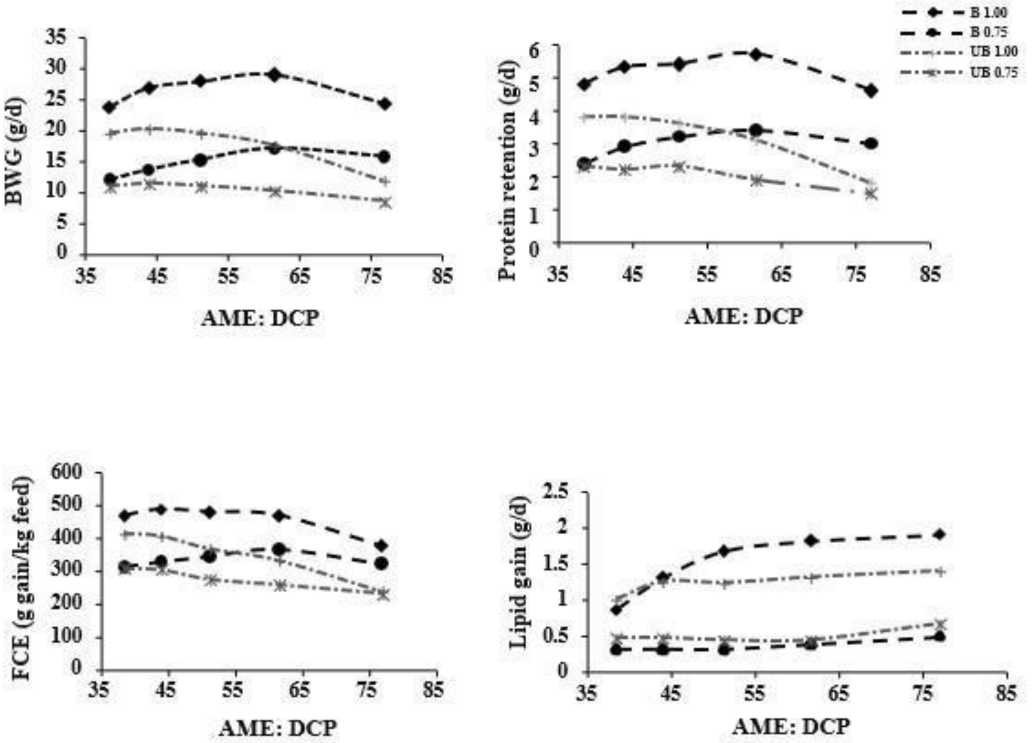

Figure 2. 4 The effect of different apparent metabolizable energy (AME) and digestible crude protein (DCP) ratios on broiler chickens' live performance (Feed conversion efficiency, FCE and body weight gain, BWG) and carcass parameters. The birds were either offered a balanced protein diet (B) or an unbalanced protein diet (UB) at two levels of feeding 1.00 (ad lib) and 0.75 (restricted), respectively. Data were obtained from (Gous *et al.*, 2018).

Amino acids are considered to have anabolic effects on protein synthesis (Yuan *et al.*, 2015; Sato *et al.*, 2016); thus, it is commonly understood that there is a positive correlation between dietary protein, live performance, and carcass composition traits in broiler chickens (Sharma *et al.*, 2018). Dozier *et al.* (2007) noted that increasing ME from 13.47 to 13.85 MJ/kg improved FCR (4.5%) and yielded higher abdominal fat (10.4%), but there were no significant differences in BWG or carcass weight (Table 2.7). Broilers that were offered a diet with moderate ME density (13.56 MJ/kg) and high AA levels (18%CP, 0.98% Lys and 0.83% TSAA) produced heavier carcasses, while lower AA densities had opposite results. During this trial, the diets were fed from 42 to 56 days of age. Johnson *et al.* (2020a) tested the response of Cobb 500 X MV broiler chickens to varying AA levels (1.11, 1.19 and 1.28% total Lys) at low (12.81MJ/kg) and high (13.27 MJ/kg) ME densities. The broilers offered lower total Lys-high ME were significantly less heavy at 26 days, and low AA and ME (main effects) resulted in significantly low FCR values. There was also an inverse relationship between total Lys and abdominal fat yield, while there was direct proportionality between dietary energy and abdominal fat. Overall, the authors observed better broiler responses to high AA and ME concentrations. Therefore, it can be inferred that when feeding high-energy diets to broilers, the concentration of dietary AA should be raised proportionally to improve performance and carcass composition (Chen *et al.*, 2019; Barekatain *et al.*, 2021b).

Even though higher digestible Lys levels may improve performance, white striping in breast meat has also been shown to be more prevalent with such diets, particularly in high-yielding broiler strains (Petracci *et al.*, 2013; Lorenzi *et al.*, 2014). Cruz *et al.* (2017) fed a basal diet with graded increments of 0.08% digestible Lys from 0.77% to 1.17% and from 0.68 to 1.07% (at 13.0 M/kg ME) for grower and finisher phases, respectively. While there was a significant improvement in performance and carcass measures with the increments, it also correlated with the induction of white striping and woody breast, with prevalence at 32.3 and 85.9% and 87.1 and 89.2%, respectively, for the corresponding phases. From the perspective of other AA, most authors point to positive responses when optimal ideal (balanced) protein ratios are maintained (Panda *et al.*, 2011). Figure 2.5 illustrates the implications of unbalanced feed protein. Mirzaaghatabar *et al.* (2011) indicated that at standard ME to protein ratios, dietary Met levels of 1.2 and 0.90% for starter and grower significantly improved performance and revealed some immunological benefits, such as the significant elevation of antibody activity. This may enhance live performance and reduce mortalities in the sometimes-stressful commercial conditions (Baracho *et al.*, 2019).

While several broiler breeder companies publish nutrient specifications (with set nutrient ratios) for producers and feed formulators, some researchers argue that constant adjustments are necessary; therefore, these should be used as a guide and not as the only standard. The adjustments could be necessitated by factors such as the need to keep up with the requirements of modern broiler strains in diverse production conditions, reduce broiler production environmental footprint and the ever-pressing issue of ingredient prices and availability, which may influence diet formulation (Sorathiya *et al.*, 2014; Wu *et al.*, 2022). Karomy *et al.* (2019) examined the suitability of breeder recommendations for dietary ME and CP densities for producers in the Basrah region, Iraq. They determined that for optimal performance, the dietary nutrient densities should change from 23% CP at 12.51 MJ/kg to 24% CP at 13.05 MJ/kg ME maintaining a constant ME: CP ratio (54 MJ/kg protein) for the starting phase, and from 19% CP at 12.72 MJ/kg ME to 20% CP at 13.41 MJ/kg ME, also maintain a constant ratio (67 MJ/kg protein) for the finisher. The geographical location specification could signify the influence an area or region could have on the nutrient needs of broiler chickens, which in the case of this Middle Eastern nation could be the issue of ambient temperature, amongst others, since they usually experience one of the hottest average daily temperatures (Morshed *et al.*, 2018). Maharjan *et al.* (2020) noticed quadratic trends for tested parameters performance in a range of AA densities (80 to 120 at 10% increments, as per *Evonik Amino Chick®* recommendations (Evonik, 2020) at constant 13.18 MJ/kg ME in hot (HT) daily average 29.61ºC or cool (CT) daily average 22ºC conditions. The latter performed significantly better overall**.** Optimum responses for average daily gain (ADG) were 89.72 g HT and

113.44 g/d CT occurring at AA concentrations 120 and 109.5%, respectively, while those for FCR in the respective conditions were 1.79 and 1.58 at AA levels 120 and 117.5%, respectively.

Attia and Hassan (2017) evaluated the effect of different dietary ME and CP levels on the performance of broiler chickens exposed to chronic heat stress. All birds except the control group were kept under 36 ± 3ºC (40-60% relative humidity) for four continuous days per week from 28 to 49 days of age. The broilers which were fed a high (13.8MJ/kg) ME and high (22%) CP had similar BWG to the control group and a significantly higher value than those fed a normal 13.2MJ/kg ME, low (19%) and high (22%) CP. The normal ME and low CP group had the worst FCR, while the high ME and high CP did not differ significantly from the control group. Also, from the same study, the high ME and high CP group had noticeably lower percentage weight values for the proventriculus, while the normal ME high CP had significantly higher intestine and liver (%) values, potentially implying better nutrient uptake and utilisation. No significant differences were observed between all groups for abdominal fat yield and other organs, such as the gizzard and heart. There was also no difference among treatments in meat physical traits (i.e. pH and tenderness) and the chemical composition of the meat except for lipid content, where the normal ME high CP group had a noticeably higher value.

Attia *et al.* (2020) demonstrated that it is possible to reduce CP from 18 to 15% for diets enriched with critical AA (i.e. Lys and Met) when finishing broiler chickens (28-49 days of age) at 12.59 MJ/kg ME control and 12.55 and 12.72 MJ/kg ME for treatment diets in less conducive hot climatic conditions (34±6ºC average temperature and 54±9% relative humidity). The low-CP diets lowered N excretion by up to 21% without adversely impacting performance or final carcass weights and quality measures. In contrast, Chrystal *et al.* (2020) discussed that in a range of isocaloric (13.10MJ/kg) diets fed from 14 to 35 days, reducing CP from 21 to 18% CP can be achieved with no adverse effects on broiler performance; however, going as low as 16.5% negatively affected performance. For instance, FCR and abdominal fat increased significantly from 1.55 to 1.608 and 0.864 to 1.46%, respectively. The authors also reported that increasing the density of free (unbound) AA did not improve performance at the lowest CP tested. These contrasting findings could be an indication that such measures (widening the ME: CP ratio) may be particularly viable in longer production cycles as the birds could gradually adapt to such rations. Also, it could demonstrate the alteration in metabolic activity and dietary responses of broiler chickens in hot environments compared to thermoneutral conditions (Donkoh, 1989), hence the need for changing or adapting nutrient specifications.

Literature also indicates that gender may influence broiler responses to varying dietary ME density and CP levels, with potential implications on production parameters such as growth performance and meat quality parameters (Shahin & Abd El Azeem, 2006; Taghinejad-Roudbaneh *et al.*, 2011; Mutibvu *et al.*, 2020). And as earlier reported by Robbins (1981), the distinction between the sexes may be more evident in the heavier breeds. Corzo *et al.* (2005) described the influence of dietary AA density and gender on the growth and carcass traits of broiler chickens. The diets comprised of low and high digestible Lys levels (1.09%; 1.22%), (0.97; 1.07%), (0.84; 0.92%) and (0.79; 0.81%) at constant ME (12.87, 12.99, 13.39 and 13.56 MJ/kg) for starter, grower, finisher, and post-finisher phases respectively. Male broilers showed significantly better BWG values than their female counterparts even though females had higher FCR values, and the mortality rate was not affected by either variable. The birds (both sexes) fed high AA density showed significantly lower fat accumulation and yielded heavier weights for breast meat and tenders. Females had a significantly high-fat percentage, wings, drum, and saddles yields. In the case of gender, the most significant concern might be the biological inclination of female broilers to accumulate more fat than their male counterparts (Benyi *et al.*, 2015). However, from the above discussion, it appears possible to mitigate excessive fat accretion through dietary ME and CP (AA) optimisation, even though this might be better archived in sex-separated flocks.

2.7 Conclusions

The broiler meat sector is one of the most valuable contributors to food security in most developed and developing countries; thus, ensuring its viability is crucial. Dietary ME and CP are jointly the greatest feed constituents and cost factors in broiler production, influencing broiler performance significantly. Broiler responses (i.e., BWG and FCE) in a range of ME: CP ratios vary as some may exhibit linear or non-linear patterns. Generally, high ME will usually decrease FI and exacerbate excessive fat deposition. At the same time, dietary CP/AA density is directly proportional to carcass and breast meat yield. Overall, a proportionate adjustment in both ME and protein will result in the best broiler responses. Dietary energy, and protein imbalances can have significant implications on production. And the factors affecting broiler response to different energy and protein ratios are dynamic (e.g. bird strain and heat stress). Thus, understanding the related consequences of these anomalies is also pivotal to diet optimization and ensuring good broiler performance.

Chapter 3

The effect of dietary nitrogen-corrected apparent metabolizable energy on feed intake and live performance of Cobb 500 broiler chickens

Abstract

The effect of incremental changes in dietary AMEn while maintaining a constant dLys: AMEn ratio was assessed. A total of 1440 unsexed day-old Cobb 500 broiler chicks were randomly allocated to 48 pens (i.e. 30 chicks per pen) in a completely randomized block design. Experimental diets were formulated to contain eight different dietary AMEn concentrations ranging from 11.30 to 13.05 MJ/kg (+0.25MJ/kg), while adjusting dLys levels to maintain a constant dLys: AMEn ratio of 0.97. The broilers were fed a standard broiler starter diet for a period of 14 days, whereafter the experimental diets were fed until the end of the trial period (i.e. Day 35). Feed intake decreased linearly with incremental changes in dietary AMEn (P<0.05). Dietary AMEn intake and energy efficiency ratio (EER) differed significantly between treatments (P<0.05). Dietary AMEn increased linearly (P<0.05) with incremental changes in AMEn, while EER exhibited a quadratic response. No differences in BWG were observed for the overall period (14-35 days) (P>0.05). According to the response curve obtained ($Y = -58.952x^2 + 1473.9 - 6937.9, P < 0.05$), BWG was optimised at 12.55 MJ/kg. Increasing AMEn while maintaining the dLys: AMEn ratio linearly improved FCR (P<0.05), and significant differences occurred between the lowest and the highest nutrient densities. It can be concluded that broilers can sustain optimal growth performance in a range of dietary AMEn concentrations if an ideal balance between dietary AMEn and nutrients is maintained.

Keywords: apparent metabolizable energy, broiler, energy concentration; digestible lysine: energy ratio; performance

3.1 Introduction

Broiler diet formulation is primarily based on energy requirements to optimise performance as well as economic and sometimes environmental aspects of modern production. Energy is the primary driver of growth in poultry because it powers cellular activities and whole-body growth and development (Hu *et al.*, 2021). So, throughout the production cycle, broilers must consume enough feed to maintain a positive energy balance. Apparent metabolizable energy (AME) is the broadly accepted metric that reflects the energy concentration in feed ingredients and poultry diets. In the ME system, the amount of metabolically available energy is calculated as the total energy of the feedstuff or compound feed minus the total energy of the excreta, resulting in AME generally measured in megajoules (MJ) per kg of feed material (Wu *et al.*, 2020). Apparent metabolizable energy can be further modified by correcting for zero-N retention; the resultant is denoted by AMEn. However, there are contradictory ideas on whether nutritionists should use AME or AMEn in diet formulation. Some investigators point out that AMEn provides the best reflection of dietary ME (Lopez & Leeson, 2007), while others argue that AMEn may underestimate ME values of some high-protein ingredients (Abdollahi *et al.*, 2021). Since no conclusive findings have been reached thus far, dietary energy concentration in broiler diets may be formulated based on AME or AMEn values.

Several studies have demonstrated that broiler FI varies (i.e. increase or decrease) according to dietary energy concentration. Therefore, it may be regarded as a biological controller of broiler FI, which could also determine the intake of other dietary nutrients (Liu *et al.*, 2016; Classen, 2017; Liu *et al.*, 2019). Physiologically, it has been established that energy intake level influences the broilers' protein metabolism (Swennen *et al.*, 2004; Musigwa *et al.*, 2020). Protein intake determines nitrogen (N) balance if the diet supplies sufficient energy to satisfy the bird's biological needs (Musigwa *et al.*, 2020). At the same time, if the diet supplies adequate protein (N and essential AA), the level of energy intake can also influence N balance and subsequently impact broiler growth rate (Lamot *et al.*, 2017; Musigwa *et al.*, 2020). It is also known that if the diet does not contain sufficient energy or nutrients such as lysine (Lys), an increased intake of either, above the required level will adversely affect feed efficiency (Abdel-Hafeez *et al.*, 2016; Ahiwe *et al.*, 2018; Gous *et al.*, 2018). Therefore, maintaining the ideal balance between energy and nutrients in broiler nutrition is fundamental for promoting optimal growth and feed utilization.

Rosa *et al.* (2007) assessed the influence of energy intake on the performance of different genetic lines of broilers, i.e. the improved (AgRoss 308) and an unimproved (PCLC-Embrapa) strain. Throughout the two-phase cycle, diets contained either 12.34, 13.39, or 14.43 MJ/kg ME. The CP content for all phase one (1-21 days) diets was 21.5%, and phase two (22-42 days) all contained 19% CP. Feed intake was equally restricted daily for all treatment groups to impose a range of energy intakes. Significantly higher body weight gain (BWG) and better FCR were recorded for the diet containing high energy (i.e. higher energy intake) level, and lower values were observed in groups that were fed the lowest energy level (i.e. low energy intake). The improved line performed significantly better overall, indicative of enhanced energy metabolism and nutrient utilisation in modern broilers. Thus, the optimal dietary energy concentration that will meet the demands of high-producing modern broilers must be determined.

This study aimed to assess the effect of incremental changes in dietary AMEn while maintaining a constant dLys: AMEn ratio to determine the most suitable AMEn density. It was expected that the broilers would adjust their performance based on the diet provided in the 14-to-35-day experimental period. Thus, the hypothesis tested was that FI would decrease while growth performance would improve with incremental changes in dietary AMEn concentration at a constant dLys: AMEn ratio.

3.2 Methods and materials

3.2.1 Study site

The experiment was conducted with the approval of the University of KwaZulu's Natal Animal Ethics Committee (Reference number: AREC017/018). It was conducted at the Poultry Section of Ukulinga Research Farm, Pietermaritzburg, KwaZulu-Natal Province, South Africa. The farm is located at 30°24' south; 29°24' east, at an altitude of about 700 meters.

3.2.2 Birds and housing

A total of 1440 unsexed day-old Cobb 500 broiler chicks were reared in an environmentally controlled house and were randomly allocated to 48 pens (3 x 2m) ; each containing 30 chicks per pen (i.e. 0.2 m^2 floor space/chick). The trial housing facility was thoroughly cleaned and disinfected a week before the experiment commenced. The floor in each pen was covered with approximately 10cm wood shavings. The bedding was turned once or twice a week or changed when necessary. The temperature was initially set to 31.5ºC and was decreased gradually by 1-1.5ºC each day after to a final average temperature (21ºC). After the initial 24-hour continuous lighting, the 23 Light:1 dark (Day 1 to 7) and 16 Light:8 dark (Day 7 to 35) lighting programs were introduced.

3.2.3 Experimental diets and feeding

Treatments consisted of eight dietary AMEn concentrations and six replications per treatment. Forty-eight experimental pens were divided into 6 blocks and within each block, pens were randomly assigned to one of the eight experimental treatments. Dietary AMEn was increased by +0.25 MJ/kg from 11.30 to 13.05 MJ/kg while adjusting dLys to maintain a constant 0.97 dLys: AMEn ratio. The ingredient and nutrient composition of the dietary treatments is presented in Table 3.1.

All treatments included AA based on the ideal AA concept, where other essential AA are expressed relative to Lys requirements. The chicks were started with the same commercial starter crumbles, and from 14 days of age, the respective experimental diets were introduced and fed to depletion (35 days). The chicks were initially fed with open trays (two per pen). After seven days, two feeders per pen were used. Water was provided through nipple drinkers. The height for both the self-feeders and drinkers was adjusted with the age of the birds. The broilers had unlimited access to feed and water.

Table 3. 1 The ingredient and calculated nutrient composition of broiler diets formulated for varying apparent metabolizable energy (AMEn) densities at a constant dLys: AMEn ratio (experiment one).

	Treatment							
Ingredients (%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Maize	62.53	63.17	60.53	58.58	56.67	54.01	51.56	48.88
Soya Hi Pro (46%+)	25.35	28.36	29.74	30.5	31.25	32.63	33.44	34.85
Sunflower Oilcake	6.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Gluten meal 20 %	2.10	-	-	-	-	-	-	-
Soya Oil	0.58	1.06	2.33	3.47	3.62	3.89	4.71	5.98
Soya Oil PPA	-	-	-	-	1.0	2.0	2.5	2.5
Limestone	0.8	0.8	0.79	0.69	0.67	0.58	0.56	0.57
Bio-lysine 70%	0.51	0.47	0.47	0.47	0.48	0.48	0.49	0.49
Valine 10% Dilution	0.51	0.5	0.51	0.61	0.61	0.72	0.73	0.73
MCP^1	0.36	0.39	0.38	0.37	0.36	0.35	0.35	0.33
NaCl fine	0.31	0.31	0.31	0.31	0.31	0.31	0.31	0.31
DL-Methionine	0.3	0.31	0.33	0.34	0.35	0.37	0.38	0.39
Vit/Min premix^2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
L-Threonine	0.14	0.14	0.14	0.15	0.16	0.16	0.16	0.17
ARG90 VAL10 25% DIL	0.12	0.11	0.1	0.12	0.13	0.12	0.13	0.11
Choline Chloride 75%	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11
Clinacox 50% dilution	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
Zinc Bacitracin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Duracube	-	-	-	-	-	-	0.3	0.3
Total	100	100	100	100	100	100	100	100
Nutrient composition (%)								
Moisture	11.73	11.72	11.55	11.4	11.25	11.08	10.87	10.7
AMEn (MJ/kg)	11.3	11.55	11.8	12.05	12.3	12.55	12.8	13.05
Starch	38.94	39.01	37.43	36.25	35.1	33.45	32.02	30.41
CP	19.36	19.59	20.04	20.29	20.52	20.99	21.21	21.67
Lys^3	1.10	1.12	1.15	1.17	1.19	1.22	1.24	1.27
Met^3	0.57	0.58	0.59	0.61	0.62	0.65	0.66	0.67
TSAA^3	0.83	0.84	0.86	0.88	0.89	0.92	0.93	0.95
Thr^3	0.75	0.76	0.78	0.8	0.81	0.83	0.84	0.86
Arg^3	1.16	1.18	1.21	1.23	1.25	1.28	1.3	1.33
Trp^3	0.19	0.2	0.21	0.21	0.21	0.22	0.22	0.23
Val^3	0.83	0.84	0.86	0.88	0.89	0.92	0.93	0.95
Ile^3	0.7	0.72	0.74	0.75	0.76	0.78	0.79	0.81
Leu^3	1.43	1.46	1.48	1.49	1.5	1.52	1.52	1.55
Gly + Ser^3	1.47	1.49	1.53	1.54	1.56	1.6	1.61	1.65
EB^4	204	212	218	222	225	231	234	240
Fat	3.0	3.44	4.6	5.67	6.74	7.9	9.12	10.29
CF	4.17	3.67	3.64	3.6	3.57	3.54	3.49	3.46
Ash	5.15	5.15	5.19	5.22	5.24	5.29	5.31	5.36
Ca	0.65	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Total P	0.52	0.51	0.51	0.51	0.51	0.51	0.51	0.51
dLys: AMEn	0.97	0.97	0.97	0.97	0.97	0.97	0.97	0.97

1 Monocalcium Phosphate **2** supplied per kg, vitamin A (10000IU), vitamin D3 (10000IU), vitamin E (50mg), vitamin k (4mg), vitamin B1 (5mg), vitamin B2 (8mg), vitamin B6 (4mg), vitamin B12 (0.02mg), folic acid (2mg), niacin (50mg), calcium pantothenate (20mg), choline (700mg), biotin (0.25mg), Mn (120mg), Zn (110mg), Cu (10mg), Se (0.3mg). **3** digestible; **4** Electrolyte balance (meq/kg)

3.2.4 Measurements

Initial body weights (BW) were measured on the day of arrival. The average body weight (BW) per pen was measured by bulk weighing and dividing the overall BW by the number of broilers in the pen. The weighing was carried out on a weekly until the end of the experiment at 35 days of age. The average body weight gain (BWG) per interval was determined using equation 1. Average feed intake (FI) was calculated as the difference between the feed allocated and that remaining at the end of each week, divided by the number of birds in the pen during each period. Feed conversion ratio (FCR) was calculated by dividing the average FI by BWG. The average AMEn intake was determined using equation 2, and the energy efficiency ratio **(**EER**),** g gain/MJ AMEn, was determined by dividing BWG by AMEn intake.

$$\mathrm{BWG} = [\text{final ave.}\,\mathrm{BW} - \text{initial ave.}\,\mathrm{BW}] \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\text{AMEn intake} = [({}^{\mathrm{FI}}/_{1000}) * \text{dietary AMEn}] \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

3.3 Statistical analysis

The experiment had a completely randomised block design. Data were analysed using the analysis of variance function from 23rd edition of GenStat (VSN, 2023). The differences between means were determined using Tukey's method. Linear and polynomial regressions were performed to determine the response curves for different parameters. Significance was set at 5%.

3.4 Results

During this trial, mortality rate was unaffected (P>0.05) by the dietary treatments. The responses of Cobb 500 broilers subjected to different dietary AMEn concentrations while adjusting dLys levels to maintain a constant dLys: AMEn ratio are given in Table 3.2. Feed intake differed in all three weekly phases and the overall growth period (P<0.05), decreasing linearly with the increase of dietary AMEn (Figure 3.1). Dietary AMEn intake and EER differed significantly between treatments (P<0.05). Dietary AMEn intake? increased linearly with incremental changes in nutrient density, while EER exhibited a quadratic response (Figure 3.2). During the first week (14-21 days), broilers that were fed diets 5 to 8 had higher body weight gain compared to those that were fed diet 1 (P<0.05). However, no significant differences were observed for mean BWG in the subsequent weeks and the overall growth period (14-35 days) (Table 3.2). Although, there were no differences between means, there was a significant, increasing quadratic response in BWG (Figure 3.3). Increasing AMEn while maintaining the dLys:

AMEn ratio improved FCR ($P<0.05$), with significant differences occurring between the lowest and the highest nutrient densities (Table 3.2). Feed conversion ratio decreased linearly with increasing dietary AMEn (Figure 3.4**)**.

Table 3. 2 Performance parameters^{2} of broilers subjected to different AMEn concentrations at a constant dLys: AMEn ratio from 14 to 35 days of age^{1}.

	Dietary treatment									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	SEM	*P-value*
14-21d										
FI	811.0^{c}	816.8^{c}	786.5^{abc}	772.4^{abc}	776.5^{abc}	803.9^{bc}	755.8^{ab}	744.7^{a}	14.82	<0.001
BWG	556.8^{a}	597.3^{ab}	595.6^{ab}	582.6^{ab}	603.5^{b}	628.6^{b}	609.1^{b}	607.9^{b}	14.51	0.002
FCR	1.46^{c}	1.36^{bc}	1.32^{ab}	1.33^{ab}	1.28^{ab}	1.26^{a}	1.24^{a}	1.23^{a}	0.014	<0.001
21-28										
FI	1142^{cd}	1165^{d}	1128^{bcd}	1113^{abcd}	1104^{abc}	1138^{bcd}	1080^{ab}	1062^{a}	18.97	<0.001
BWG	787.0	797.7	813.7	796.8	812.2	817.6	815.6	813.0	17.7	0.585
FCR	1.45^{d}	1.46^{d}	1.38^{c}	1.39^{c}	1.36^{abc}	1.39^{c}	1.32^{ab}	1.29^{a}	0.012	<0.001
28-35										
FI	1358^{c}	1359^{c}	1308^{bc}	1308^{abc}	1277^{abc}	1241^{ab}	1258^{ab}	1216^{a}	28.49	<0.001
BWG	834.8	840.9	847.4	871.9	842.2	835.3	851.1	832.8	30.4	0.939
FCR	1.62^{d}	1.61^{d}	1.54^{c}	1.50^{c}	1.52^{bc}	1.49^{abc}	1.48^{ab}	1.46^{a}	0.013	<0.001
14-35d										
FI	3311^{cd}	3340^{d}	3222^{bcd}	3194^{bc}	3157^{ab}	3182^{bc}	3094^{ab}	3023^{a}	43.88	<0.001
AMEn intake	37.41^{a}	38.58^{ab}	38.02^{ab}	37.81^{ab}	38.83^{ab}	39.94^{b}	39.60^{b}	39.45^{ab}	0.62	0.001
EER	58.30^{ab}	57.94^{ab}	59.35^{b}	58.57^{ab}	58.14^{ab}	57.13^{a}	57.47^{ab}	57.14^{a}	0.607	0.011
BWG	2179	2236	2257	2251	2258	2282	2276	2254	41.01	0.312
FCR	1.52^{d}	1.50^{d}	1.43^{c}	1.42^{c}	1.40^{bc}	1.40^{bc}	1.36^{ab}	1.34^{a}	0.015	<0.001

a-d means with different superscripts within a row differ significantly ($P < 0.05$);**1** Mean feed intake (FI) g, nitrogen-corrected apparent metabolizable energy (AMEn) intake (MJ/bird), energy efficiency ratio (EER) (g gain/MJ), body weight gain (BWG) g, and feed conversion ratio (FCR); 2 diet 1 to 8 contain 11.30 MJ/kg AMEn & 1.10% dLys; 11.55MJ/kg & 1.12% dLys; 11.8 & 1.15% dLys; 12.05 MJ/kg & 1.17% dLys; 12.3 MJ/kg AMEn & 1.19% dLys; 12.55 MJ/kg AMEn & 1.22% dLys; 12.8 MJ/kg AMEn & 1.24% dLys; 13.05 MJ/kg AMEn & 1.27% dLys, respectively.

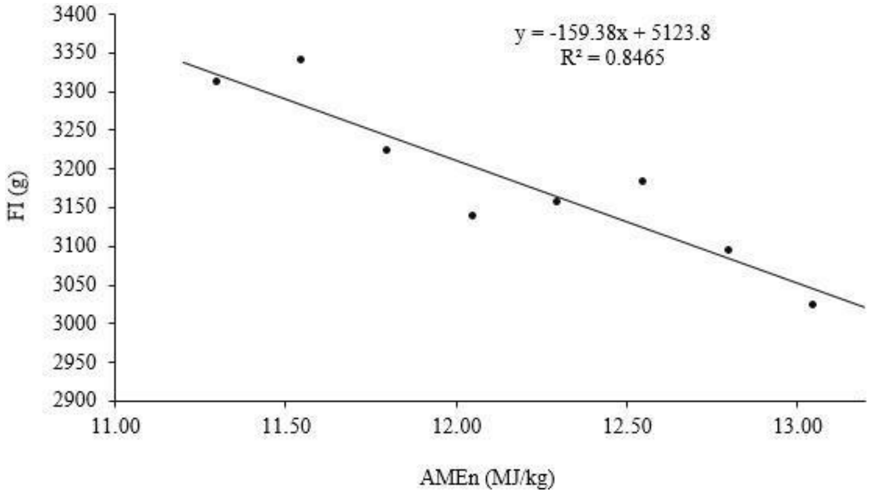

Figure 3. 1 The effect of increasing dietary nitrogen corrected apparent metabolizable energy (AMEn) concentration at constant digestible lysine (dLys): AMEn ratio on feed intake (FI) from 14 to 35 days of age (*P*<0.001).

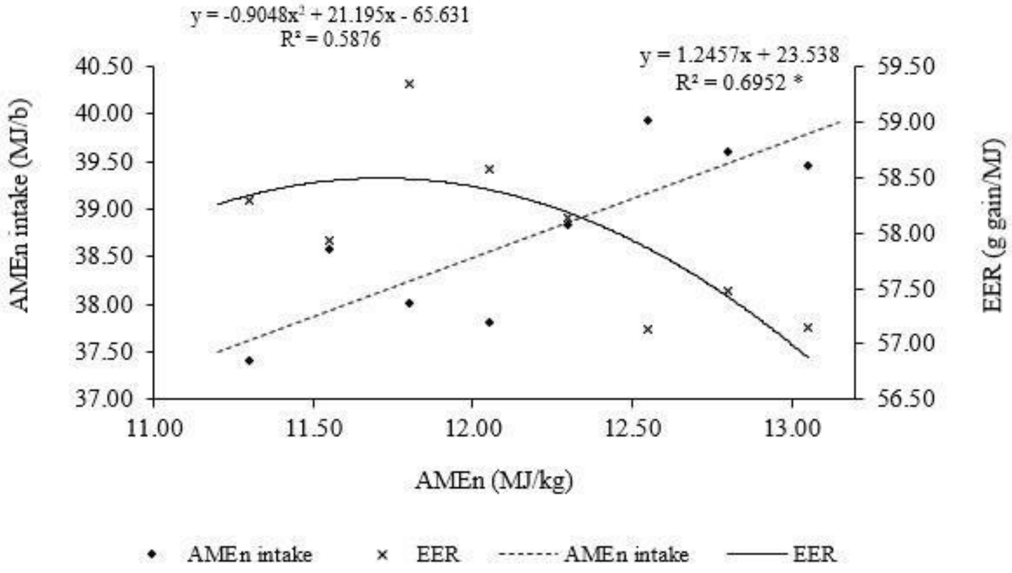

Figure 3. 2 The effect of increasing dietary nitrogen corrected apparent metabolizable energy (AMEn) concentration at constant digestible lysine (dLys): AMEn ratio on AMEn intake (* *P*<0.05) and energy efficiency ratio (EER) from 14 to 35 days of age.

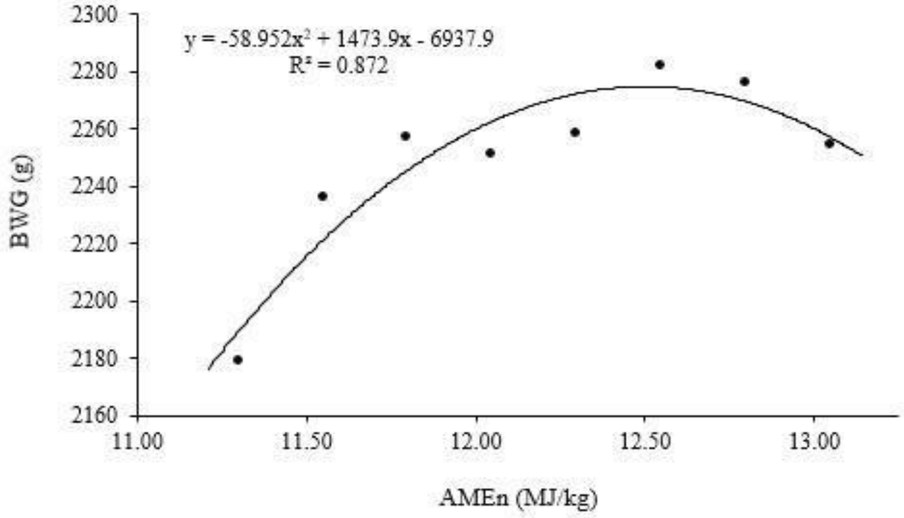

Figure 3. 3 The effect of increasing dietary nitrogen corrected apparent metabolizable energy (AMEn) concentration while maintain a constant digestible lysine (dLys): AMEn ratio on body weight gain (BWG) from 14 to 35 days of age (*P*<0.05).

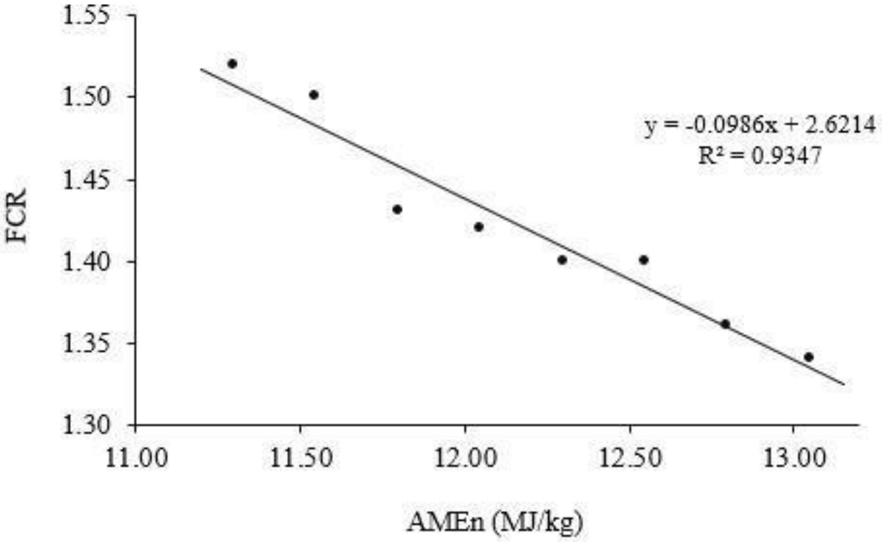

Figure 3. 4 The effect of increasing dietary nitrogen corrected apparent metabolizable energy (AMEn) level at constant digestible lysine (dLys): AMEn ratio on feed conversion ratio (FCR) from 14 to 35 days of age (*P*<0.001).

3.5 Discussion

For the past decades, the broiler industry has achieved a significant improvement in genetic potential, resulting in higher growth rates than previously possible (Marcu *et al.*, 2013; Haque *et al.*, 2020). Live performance must be prioritised while keeping in mind the practical consequences of diet composition and its influence on overall production (Hirai *et al.*, 2020). Literature points out that dietary AMEn significantly influences broiler responses and the efficiency of feed utilisation (Boekholt *et al.*, 1994; Barekatain *et al.*, 2021b), and it is one of the major cost factors in broiler feed (Alhotan, 2021). Feed formulation parameters include digestible AA, AMEn, and specific nutrient content. Compared to the present formulation based on defined values per unit of feed mass, a balanced nutrient density concept based on nutrient density and a balanced amino acids-to-AMEn ratio may offer more flexibility in optimising overall broiler production (i.e. economics and growth performance). Therefore, this study assessed the effect of increasing dietary AMEn while proportionally increasing dLys levels to maintain a constant dLys: AMEn ratio on live performance of Cobb 500 broiler chickens to determine the optimal AMEn and nutrient density.

The dietary treatments significantly influenced FI, AMEn intake, EER, BWG and FCR. However, at 21-28 and 28-35-day, and the overall period BWG, there were not significant differences between treatments (*P*>0.05). The observed linear decline in response to increasing AMEn suggests an inverse relationship between dietary AMEn and broiler FI. Mansilla *et al.* (2022) evaluated the effect of adjusting dietary AME while maintaining a constant SID Lys: AME ratio on the performance of Ross 308 broilers. Dietary AME and SID Lys positive controls 12.13 MJ/kg AME and 1.13% SID Lys (9-20 days), 12.34 MJ/kg AME and 1.05% SID Lys (20-31 days) and 12.55 MJ/kg AME and 1.0% SID Lys (31-42 day) were adjusted by -4, -8 and -12% to give three treatments per phase. In each phase, the constant ratio was 0.97, 0.89 and 0.83 SID Lys: AME, respectively. They reported a linear increase in FI as AME decreased at 21-31 days, 31-24 days and overall period (0-42 days). However, there were contradictory findings with regard to the Grower 1 phase. Even though the SID Lys: AME ratio reported in Grower 1 diets is the same as that used in the current study (0.97), they found no significant differences in FI. This may be due to genetic differences (i.e. energy requirements) between the broiler strains (Cobb 500 vs Ross 308) which could have influenced broiler responses to dietary AME at that specific growth and development stage, consequently impacting FI differently.

Evaluating the physiological influence of dietary AME on the FI of broiler chickens, Hu *et al.* (2019b) observed a reduction in the appetite of broilers that were fed a high ME (14,64 MJ/kg) diet. They discussed that the ME-dense diet downregulated AMP-activated protein kinase (AMPK) enzyme signalling, while the low dietary ME (12.13 MJ/kg) resulted in the opposite effect. The enzyme AMPK is primarily responsible for promoting the intake of energy-yielding substrate, such as glucose and fatty acids; therefore, it plays a crucial role in energy balance within the body of broiler chickens (Kola *et al.*, 2006; Hu *et al.*, 2019b). So, optimizing dietary energy concentration is crucial for managing FI in broilers to ensure efficient feed utilisation and improving overall growth performance.

The level of energy intake is fundamental for achieving optimal broiler growth performance (Johnson *et al.*, 2020). In the current study, the highest AMEn intake values were observed in the high AMEn diets and the lowest in the low AMEn diets**.** Mansilla *et al.* (2022) also reported that the calculated total AME intake during the duration of the experiment decreased linearly ($P<0.05$) when the dietary AME concentration was reduced. This suggests a directly proportional association between dietary AMEn and broiler FI, and this could be because other factors, such as gut capacity, may also limit AMEn intake in lower-density diets.

Energy efficiency ratio differed significantly between treatments; specifically, diets 6 and 8 recorded the lowest EER values, while the highest was observed in diet 3. Early work by Waldroup *et al.* (1976) indicated that broilers that were fed energy-dense diets consumed up to 30% more AME overall, yet they gained weight with no adverse effects on energy efficiency; AME ranged from 12.43 to 15.65 MJ/kg, and the AA (i.e. Lys) and other essential nutrients were adjusted proportionately to the dietary energy increase. This may suggest that selection for increased energy and nutrient efficiency could be reaching its peak limit in current broiler lines; thus, higher AMEn intakes may not enhance performance further (Tallentire *et al.*, 2018).

The increasing quadratic response of BWG shows that broilers fed diets with excessively high AMEn levels may exhibit diminished improvements in BWG compared to those receiving low AMEn diets. This suggests that broilers may reach a physiological limit in their capacity to utilize dietary energy for growth beyond a specific AMEn concentration. Similar results were obtained by Ataei (2022), where the BWG differed significantly between treatment means (11-24 days). However, no significant differences were observed in the 21-42 days and overall period (1-42days) when Ross 308 broilers were fed incremental dietary ME levels of 11.72, 12.13, 12.55 and 12.97 MJ/kg at a constant (0.83) dLys: ME ratio. Feitosa *et al.* (2023) also observed a significant difference in BWG between treatments in an 8-to-21-day period; however, no difference was observed in the 22-to-42-day period. The dietary treatments consisted of 12.76, 13.08 and 13.39 MJ/kg AME (8-21days) and 12.97, 13.28 and 13.60 MJ/kg AME (22-42), all fed at constant dLys: AME ratios 0.94 and 0.83 for the respective growth periods. So, the observed BWG responses suggest that broilers fed lower AMEn- nutrient diets could have maintained BW by increasing FI as they grew to compensate for the deficiencies.

The linear decrease in FCR suggests an inverse relationship between dietary AMEn and FCR.

Hidalgo *et al.* (2004) obtained similar results, where lower AME density diets presented poor FCR response values (*P*<0.05) compared to the group that was fed high AME diets from 1 to 17 days of age. In their study, AME values ranged from 12.64 MJ/kg to 13.56 MJ/kg, and the Lys: AME ratio was kept constant at 0.96. Feitosa *et al.* (2023) also observed that FCR was significantly improved when broilers were fed diets with incremental changes in dietary AME and nutrient density. In contrast, Ataei (2022) found significant differences (*P*<0.05) in FCR in the grower (11-24 days) and finisher (25-42 days) phases and not in the overall growth period (1-42 days). However, during their study, broilers were fed standard diets based on commercial Ross nutritional guidelines in the first (1-10 days) and the last phase (25-42 days). So, the observed difference between treatments in the 25-to-42-day period could have been due to a carryover effect from the different dietary treatments introduced in the grower period (11-24 days). Overall, it may be expected that FCR will improve as dietary AMEn increases if the levels do not exceed the broiler energy requirements.

3.6 Conclusion

The findings of this study highlight the necessity for innovative approaches in broiler diet formulation. Specifically, attention should be given to balancing dietary energy and nutrient density, particularly with regard to dLys (digestible lysine). The observed results partially agree with the hypothesis; the overall FI, AMEn intake and FCR exhibited significant differences, while overall BWG remained unaffected by the dietary treatments.

It can be concluded that broilers can maintain BW across a range of dietary AMEn concentrations provided that an optimal dLys: AMEn ratio is maintained. Maintaining this ratio constant while adjusting dietary AMEn levels, can enhance the flexibility of broiler feed formulation. Exploring the energy efficiency ratio as an indicator of feed utilisation could prove valuable. Future research should investigate the underlying physiological mechanisms driving the observed responses and refine strategies to optimize broiler production efficiency and sustainability.

Chapter 4

Effect of varying dietary digestible lysine in isoenergetic diets on feed intake and live performance of Cobb 500 broiler chickens

Abstract

The influence of varying dietary digestible lysine (dLys) levels in isoenergetic diets was evaluated. A total of 1440 unsexed day-old Cobb 500 broiler chicks were randomly allocated to one of 48 pens, 30 chicks per pen, in a completely randomized block design. Experimental diets were formulated to contain 8 different dietary dLys levels ranging from 0.95 to 1.30% (+0.05%). Dietary nitrogen corrected apparent metabolizable energy (AMEn) concentration was kept at 12.1 MJ/kg, giving dLys: AMEn ratios 0.78, 0.83, 0.87, 0.91, 0.95, 0.99, 1.03 and 1.07. The broilers were fed a standard broiler starter diet (0-14 days), thereafter, experimental diets were introduced (14-35 days). Feed intake and AMEn intake were not affected by the incremental changes in dietary dLys (P>0.05). Energy efficiency ratio, dLys intake and dLys efficiency ratio (dLysER) differed significantly between dietary treatments (P<0.05). Digestible Lys intake linearly increased while dLysER decreased as dLys inclusion level increased (P<0.05). Overall, body weight gain (BWG) differed significantly between treatment groups and exhibited an increasing quadratic response (P<0.05). According to the response curve obtained ($Y = -1119x^2 + 2871.7x + 301.11$, $P < 0.05$), BWG was optimised at the highest (1.3%) dLys level, 1.07 dLys: AMEn ratio. Feed conversion ratio decreased linearly with increasing dLys levels (P<0.05). It can be concluded that increasing dLys (adjusting the dLys: AMEn ratio) will improve broiler responses in isoenergetic diets, however, an optimal level must always be determined.

Key words: broiler, digestible lysine, dietary energy, isoenergetic, performance

4.1 Introduction

The primary goals of the broiler industry are to achieve higher performance outputs, superior product quality, and reduce costs (Maynard & Kidd, 2022). So, there has continually been a need for improved feed efficiency and growth rates, and this is evident in the significantly improved current broiler lines (Schmidt *et al.*, 2009; Henn *et al.*, 2014; McCrea *et al.*, 2014; Prakash *et al.*, 2020). Some studies indicate that modern broilers may require higher dietary nutrient concentrations for optimum performance in contrast to earlier strains (Siqueira *et al.*, 2013; Bernal *et al.*, 2014), even though they consume less feed overall compared to the latter to reach market weight (Siegel, 2014). At the same time, some publications have reported non-linear broiler responses to increased nutrient concentrations

(Acacio *et al.*, 2020), while some even recommend levels below the NRC (1994) values (Wang *et al.*, 2016; Belloir *et al.*, 2017; Lee *et al.*, 2020b). Therefore, there is still a necessity for more precision in diet formulation and feeding strategies if the added benefit of genetic improvement in modern broilers is to be realised (Moss *et al.*, 2021).

As one of the limiting amino acids (AA), broilers require lysine (Lys) for a variety of physiological processes, primary among them being proteinogenesis, growth and maintenance (Selle *et al.*, 2023). Multiple studies have evaluated the influence of dietary Lys levels on overall growth performance and highlighted the need to adjust dietary inclusion levels with advances in broiler genetics. Bernal *et al.* (2014) observed a quadratic effect on BWG and FCR when Cobb 500 broilers were fed diets with varying dietary dLys levels, 1.06, 1.12, 1.18, 1.24, and 1.30% (10-21 days) and 0.9, 0.98, 1.04, 1.10, and 1.16% (22-35 days). Dietary AME was kept constant at 12.55 MJ/kg and 12.97 MJ/kg for the respective phases. Johnson *et al.* (2020b) evaluated the effect of varying AA densities in isoenergetic diets on the performance of Cobb 700 × MV broilers. They reported that mean BW and FCR were significantly improved ($P<0.05$) compared to the control groups. The control diets treatments consisted of 12.79MJ/kg and 1.07% dLys (12–26 days), 12.98 MJ/kg and 0.96% dLys (26-36 days), and 13.02 MJ/kg and 0.89% dLys (36-49 days). In each phase, dietary treatments were obtained by increasing dLys (as a limiting AA) levels by 5, 10, 15 and 20% of the control diet. They concluded that the positive responses support feeding increased dietary digestible essential AA levels to the Cobb 700 × MV broilers.

The level of FI determines nutrient intake, which in turn impacts overall broiler performance. Although it is commonly recognised that dietary energy significantly affects broiler FI, Liu *et al.* (2019) demonstrated that dietary AA density could also influence FI. They observed that increasing ME from 11.25 to 13.5 MJ/kg resulted in a decline in FI, while digestible Lys (dLys) ranging from 0.92 to 1.21% exhibited a quadratic effect on FI independently of dietary ME. Therefore, dietary AA levels may also be expected to influence broiler FI in a range of dietary treatments. This study aimed to evaluate the influence of varying dietary dLys levels while maintaining a constant AMEn level (12.1 MJ/kg) on broiler FI and performance. The hypothesis tested was that increasing dLys (manipulating the dLys: AMEn ratio) would significantly impact FI and enhance growth performance.

4.2 Methods and materials

4.2.1 Study site

The study site has been described in detail in section 3.2.1

4.2.2 *Birds and housing*

A total 1440 unsexed Cobb 500 broilers were used. Details of housing are described in section 3.2.2

4.2.3 *Experimental diets and feeding*

Dietary treatments consisted of eight different dietary dLys densities and six replications per treatment. Dietary AMEn density was kept at a constant level (12.1 MJ/kg) as per (CVB, 2018), and broiler dLys densities ranged from 0.95 to 1.30% at 0.05% incremental changes. The ingredient and nutrient composition of the dietary treatments is presented in Table 4.1.

All treatments included AA based on the ideal AA concept, where other essential AA are expressed relative to Lys requirements. The chicks were started with the same commercial starter crumbles, and from 14 days of age, the respective experimental diets were introduced and fed to depletion (35 days). The chicks were initially fed with open trays (two per pen). After seven days, two feeders per pen were used. Water was provided through nipple drinkers. The height for both the self-feeders and drinkers was adjusted with the age of the birds. The broilers had unlimited access to feed and water.

Table 4. 1 The ingredient and calculated nutrient composition of broiler diets formulated for varying digestible lysine (dLys) levels at constant apparent metabolizable energy (AMEn) density (12,1 MJ/kg).

	Dietary treatment							
Ingredients (%)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Maize	67.54	65.72	64.59	62.95	60.1	57.87	55.66	53.43
Soybean oil cake (46%)	20.47	22.31	24.44	27.01	29.46	31.31	33.15	35.0
Sunflower oil cake	6.0	6.0	5.16	4.01	4.01	4.01	4.01	4.01
Soya Oil	2.5	2.5	2.5	2.65	3.12	2.71	2.81	2.65
Soya Oil PPA	-	-	-	-	-	0.75	1.0	1.50
Limestone	1.28	1.28	1.04	0.94	1.02	0.97	0.92	0.84
MCP[1]	0.59	0.57	0.56	0.55	0.53	0.51	0.49	0.47
L-Lysine HCl	0.31	0.32	0.33	0.33	0.32	0.33	0.34	0.35
NaCl	0.27	0.32	0.31	0.31	0.31	0.31	0.30	0.30
DL- Methionine	0.23	0.25	0.28	0.31	0.32	0.35	0.37	0.40
Vit/Min Premix[2]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Valine 20% dilution	0.17	0.17	0.22	0.27	0.23	0.28	0.33	0.38
L-Threonine	0.11	0.11	0.12	0.14	0.14	0.15	0.16	0.16
ARG90/VAL10 dilution 25%	-	-	0.05	0.13	0.06	0.07	0.07	0.12

Sodium Bicarbonate	0.13	0.11	-	-	-	-	-	-
Choline Chloride 75%	0.11	0.06	0.11	0.11	0.11	0.11	0.11	0.11
Poulcox Monensin	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Zinc Bacitracin	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Total	100	100	100	100	100	100	100	100
				Nutrient composition (%)				
Moisture	10.99	10.96	10.98	10.96	10.87	10.8	10.74	10.67
AMEn (MJ/kg)	12.10	12.10	12.10	12.10	12.10	12.10	12.10	12.10
Starch	41.93	40.84	40.16	39.17	37.46	36.12	34.78	33.44
CP	16.92	17.74	18.12	18.92	19.99	20.74	21.48	22.22
Dig Lys[3]	0.95	1.0	1.05	1.1	1.15	1.2	1.25	1.3
Dig Met[3]	0.47	0.5	0.54	0.57	0.59	0.62	0.66	0.69
Dig TSAA[3]	0.71	0.75	0.79	0.83	0.86	0.9	0.94	0.98
Dig Thr[3]	0.65	0.68	0.71	0.75	0.78	0.82	0.85	0.88
Dig g[3]	1.0	1.05	1.1	1.16	1.21	1.26	1.31	1.37
Dig Trp[3]	0.17	0.18	0.19	0.2	0.21	0.22	0.23	0.24
Dig Val[3]	0.72	0.75	0.79	0.83	0.86	0.9	0.94	0.98
Dig Ile[3]	0.61	0.64	0.67	0.7	0.74	0.77	0.8	0.83
Dig Gly+Ser[3]	1.31	1.36	1.41	1.46	1.54	1.59	1.65	1.71
SID Lys[4]	0.97	1.02	1.07	1.12	1.17	1.22	1.26	1.31
SID Met[4]	0.48	0.52	0.55	0.58	0.60	0.63	0.67	0.70
SID TSAA[4]	0.72	0.76	0.80	0.83	0.86	0.90	0.94	0.98
SID Thr[4]	0.66	0.68	0.71	0.75	0.78	0.82	0.84	0.87
SID Arg[4]	1.02	1.07	1.12	1.18	1.23	1.28	1.33	1.39
SID Trp[4]	0.17	0.18	0.19	0.19	0.21	0.21	0.22	0.23
SID Val[4]	0.74	0.77	0.8	0.84	0.87	0.91	0.95	0.99
SID Ile[4]	0.62	0.65	0.68	0.7	0.74	0.77	0.8	0.83
SID His[4]	0.41	0.43	0.44	0.46	0.48	0.5	0.51	0.53
SID Gly + Ser[4]	1.32	1.38	1.41	1.47	1.55	1.6	1.65	1.71
EB[5]	175	175	176	185	197	206	214	223
Crude fat	4.98	4.94	4.9	5	5.38	5.66	5.94	6.22
Crude fibre	3.80	3.81	3.72	3.59	3.60	3.60	3.60	3.61
Ash	5.17	5.23	5.03	5.05	5.19	5.23	5.28	5.32
Ca	0.7	0.7	0.65	0.65	0.65	0.65	0.65	0.65
Total P	0.47	0.48	0.47	0.47	0.47	0.48	0.48	0.48
dLys: AMEn	0.78	0.83	0.87	0.91	0.95	0.99	1.03	1.07

1 Monocalcium Phosphate **2** supplied per kg, vitamin A (10000IU), vitamin D3 (10000IU), vitamin E (50mg), vitamin k (4mg), vitamin B1 (5mg), vitamin B2 (8mg), vitamin B6 (4mg), vitamin B12 (0,02mg), folic acid (2mg), niacin (50mg), calcium pantothenate (20mg), choline (700mg), biotin (0,25mg), Mn (120mg), Zn (110mg), Cu (10mg), I (4mg), Se (0,3mg);**3** digestible: **4** standardised ileal digestible **5** Electrolyte balance (meq/kg)

4.2.4 Measurements

Measurements on FI, BWG, FCR, AMEn and EER were done as describe in section 3.2.4. Digestible Lysine (dLys) intake was determined using equation 3 and dLys efficiency ratio (dLys ER) was determined by dividing BWG by dLys intake.

$$\text{dLys intake} = [({}^{\text{FI}}/_{1000}) * \text{dieatry dLys}] \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . \ (3)$$

4.3 Statistical analysis

The experiment had a completely randomised design with blocks. Data were analysed using the analysis of variance function from GenStat (23rd edition) (VSN, 2023). The differences between means were determined using Tukey's method. Linear and polynomial regressions were performed to determine the response curves for different parameters. Significance was set at 5%.

4.4 Results

During this trial, the mortality rate was unaffected (*P*>0.05) by the dietary treatments used. The mean FI, AMEn intake, EER, dLys intake, dLysER, BWG, and FCR of broilers subjected to eight different dLys levels are given in Table 4.2.

Table 4. 2 Performance parameters[1] of broilers subjected to different dLys densities at constant 12.1 MJ/kg AMEn from 14 to 35 days of age[2]**.**

	Treatment									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	SEM	*P-value*
14-21d										
FI	1030	1022	1021	1028	1031	1009	975	1004	20.47	0.185
BWG	511.5	504.7	529.2	535.9	519	542.2	531.2	551.3	19.73	0.292
FCR	2.01	2.02	1.93	1.92	1.99	1.86	1.84	1.82	0.0389	0.080
21-28d										
FI	976.9	1039.1	965.7	1008	960.5	998.8	984.3	995.1	30.69	0.281
BWG	649.1^{a}	744.7^{ab}	688.8^{ab}	728.5^{ab}	725.7^{ab}	762.6^{b}	742.3^{ab}	750.5^{b}	27.86	0.008
FCR	1.51^{b}	1.40^{ab}	1.40^{ab}	1.38^{ab}	1.32^{a}	1.31^{a}	1.33^{a}	1.33^{a}	0.0233	<0.001
28-35d										
FI	1219	1268	1215	1247	1201	1250	1193	1183	34.32	0.180
BWG	820.4	871.9	844.6	853.9	846.3	846.4	849.5	856.4	24.65	0.767
FCR	1.49^{d}	1.45^{cd}	1.44^{bc}	1.46^{bcd}	1.42^{abc}	1.48^{abc}	1.40^{ab}	1.38^{a}	0.0172	<0.001
14-35d										
FI	3227	3329	3202	3283	3192	3258	3152	3183	59.73	0.130
dLys intake	30.65^{a}	33.29^{b}	33.29^{b}	36.11^{c}	36.71^{c}	39.09^{d}	39.40^{de}	41.37^{e}	0.635	<0.001
dLysER	64.64^{e}	63.74^{e}	61.94^{e}	58.67^{d}	56.97^{cd}	55.05^{bc}	53.88^{ab}	52.15^{a}	0.8572	<0.001
AMEn intake	39.33	40.31	38.75	39.72	38.62	39.42	38.14	38.51	0.724	0.116
EER	48.31^{a}	52.63^{ab}	53.23^{b}	53.15^{b}	54.15^{b}	54.60^{b}	55.66^{b}	56.03^{b}	1.238	<0.001
BWG	1981^{a}	2121^{ab}	2063^{ab}	2118^{ab}	2091^{ab}	2151^{b}	2123^{ab}	2158^{b}	44.71	0.014
FCR	1.63^{d}	1.57^{cd}	1.55^{bcd}	1.55^{bc}	1.53^{abc}	1.52^{abc}	1.49^{ab}	1.48^{a}	0.0221	<0.001

a-e means with different superscripts within a row differ significantly (*P* < 0.05);**1)** Mean feed intake (FI) g, nitrogen-corrected apparent metabolizable energy (AMEn) intake (MJ/bird), energy efficiency ratio (EER), digestible lysine (dLys) intake (g/bird), dLys efficiency ratio (dLysER), body weight gain (BWG) g, and feed conversion ratio (FCR)

2) diet 1 to 8 contain 0.95, 1.00, 1.05, 1.10, 1.15, 1.20, 1.25, and 1.30% dLys respectively.

Mean feed intake was not affected by the incremental changes in dietary dLys (*P*>0.05) throughout the trial; the relationship between dLys inclusion level and FI is shown in Figure 4.1.

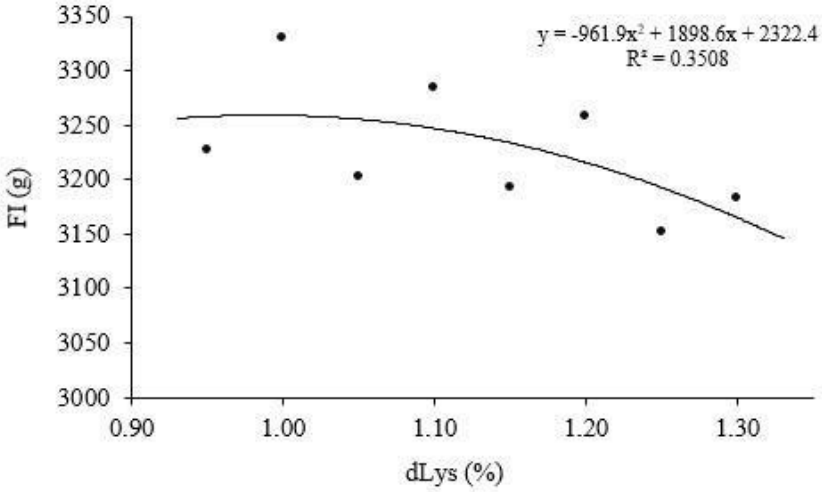

Figure 4. 1 The effect of incremental changes in digestible lysine (dLys) density at constant nitrogen corrected apparent metabolizable energy (AMEn) 12.1 MJ/kg on feed intake (FI) from 14 to 35 days of age (*P*>0.05).

There were no significant differences in AMEn intake (*P*>0.05), while EER, dLys intake and dLysER differed significantly between dietary treatments (*P*<0.05). Digestible Lys intake linearly increased while dLysER decreased as dLys inclusion level increased (Figure 4.2).

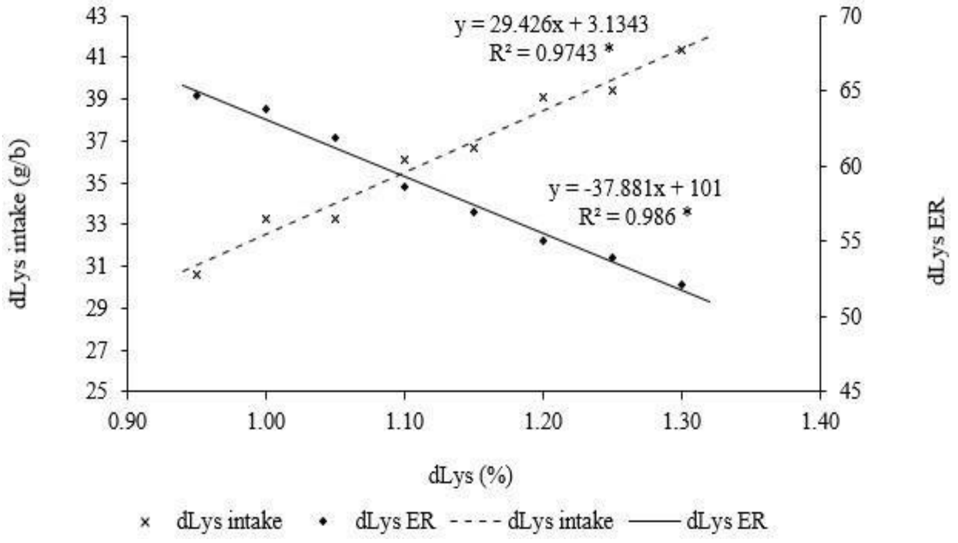

Figure 4. 2 The effect of incremental changes in digestible lysine (dLys) levels at a constant nitrogen corrected apparent metabolizable energy (AMEn) level on digestible lysine (dLys) intake and lysine efficiency ratio (Lys ER) (**P*<0.001) from 14 to 35 days of age.

During weeks one (14-21days) and three (28-35 days), BWG did not differ ($P>0.05$), while there were significant differences between treatments in week two (21-28 days) and the overall growth period (14- 35 days) ($P<0.05$); BWG exhibited an increasing quadratic response Figure 4.3.

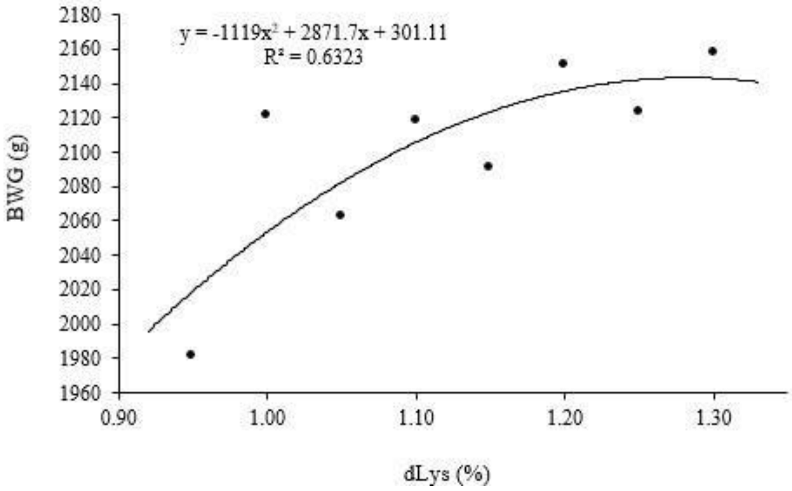

Figure 4. 3 The effect of incremental changes in digestible lysine (dLys) density at constant nitrogen corrected apparent metabolizable energy (AMEn) 12.1 MJ/kg on BWG from 14 to 35 days of age ($P<0.05$).

Feed conversion ratio differed between dietary treatments in weeks two (21-28 days) and three (28-35 days) and the overall period (14-35 days) ($P<0.05$), except in week one (14-21 days). The relationship between dLys inclusion levels and FCR is shown in Figure 4.4.

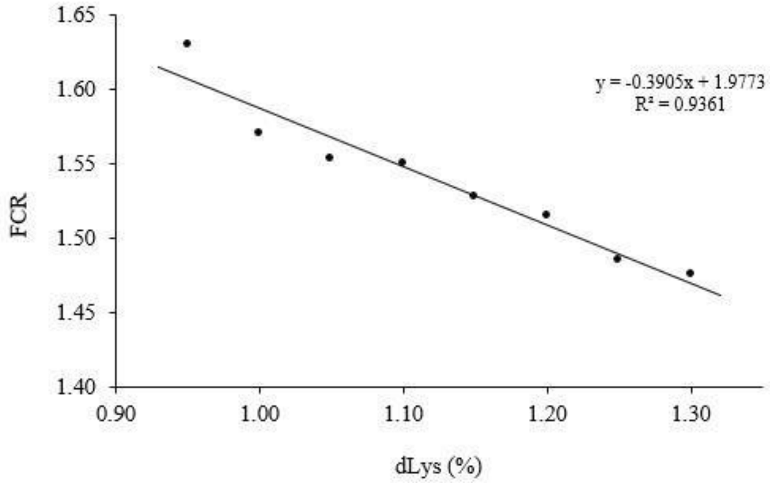

Figure 4. 4 The effect of incremental changes in digestible lysine (dLys) density at constant nitrogen corrected apparent metabolizable energy (AMEn) 12.1 MJ/kg on feed conversion ratio (FCR) from 14 to 35 days of age ($P<0.001$).

4.5 Discussion

Like other livestock sectors, the poultry industry is under intense pressure to mitigate the impact of increasing production costs (Komarek *et al.*, 2022) So, optimizing diet formulation and feeding strategies is crucial for ensuring a viable and sustainable broiler industry. The overall diet cost is influenced mainly by the broiler's nutritional requirements and the dietary ingredients (i.e. maize and soybean) utilized to meet the specifications and nutrient dense diets are more expensive (Thirumalaisamy *et al.*, 2016; Komarek *et al.*, 2022). Therefore, adjusting the nutrient specifications to maximize production is paramount. The present study aimed to evaluate the influence of varying levels of dietary dLys in isoenergetic diets on feed intake and broiler performance.

Mean feed and AMEn intake did not differ between dietary treatments (P>0.05). This observation suggests that broilers in all dietary treatments were consuming feed to satisfy their energy needs irrespective of the differences in dietary dLys levels. Despite the decline in BWG at low dLys levels (P<0.05), Zarghi *et al.* (2020) found that the FI of Cobb 500 broilers was not affected by the incremental changes in dLys levels (0.88, 0.94, 1.00, 1.06 and 1.12%) while keeping dietary AME constant at 13.28 MJ/kg. Although they observed significantly different FI values, Mansilla *et al.* (2022) also found no difference in total AME intake when the SID Lys: AME ratios were increased. Therefore, it may be argued that physiologically, dietary AMEn density imposed a significant regulatory influence (indicated by the similar FI levels across all groups since AMEn was kept constant) compared to AA density at the tested dLys levels. MacLeod (1990) mentioned that in a range of energy densities (8 to 15 MJ/kg), the control of dietary ME intake in broiler chickens took precedence over CP/AA intake, consequently influencing overall FI more significantly.

In contrast, Hirai *et al.* (2020) observed significantly higher FI values in broilers that were fed low Lys (1.00%) than those on a higher dLys density (1.18%) diet from 14 to 35 days of age. Even though increasing AME (11.25 to 13.5 MJ/kg) reduced FI independently of dLys, Liu *et al.* (2019) also demonstrated that dietary AA density (main effects) can also influence broiler FI. They observed that in a range of 0.92 to 1.21% dLys, FI exhibited a quadratic pattern. Supporting their findings, Khwatenge *et al.* (2020) reported varying physiological effects on receptor expression of hormones such as ghrelin, leptin and adiponectin that influence appetite and satiety in broiler chickens. Diets comprised different Lys densities (75%, 100% and 125% of the NRC 1994 recommended levels) at constant dietary ME (12.97 MJ/kg and 13.39 MJ/kg) and CP (23% and 22%) for the starter and grower phase, respectively. Higher Lys levels induced a negative feedback loop on receptor expression, whereas lower Lys levels had a positive effect. Thus, physiologically, there is also strong evidence supporting the theory that dietary AA density influences broiler FI (Liu *et al.*, 2019; Khwatenge *et al.*, 2020).

Energy efficiency ratio, dLys intake, and dLysER differed significantly (14-35 days). There was a significant difference in EER between treatment 1 (lowest) and the rest of the treatments except treatment 2. This observation suggests that low dLys in isoenergetic diets negatively affects the efficiency of broilers utilising AMEn for growth. In contrast, Kamran *et al.* (2011) found no significant differences in EER between the treatments containing 1.10, 1.02 and 0.90% dLys at constant AME levels of 12.24, 12.87 and 13.08 MJ/kg for the starter, grower, and finisher, respectively. Physiologically, it is known that if a diet does not contain sufficient protein (i.e. Lys), an increased AME intake above the required level will exacerbate abdominal fat deposition (Abdel-Hafeez *et al.*, 2016; Ahiwe *et al.*, 2018; Gous *et al.*, 2018). The AME concentrations Kamran *et al.* (2011) used particularly in the grower and finisher phase, were significantly higher than those used in the current study, so broilers that were low dLys could have deposited more fat, thus contributing to their BWG, and improving the calculated EER.

Barekatain *et al.* (2021b) observed a significant difference in dLys intake. The lowest value was observed in the low dLys diets, and the highest was observed in high dLys diets ($P<0.05$). However, the dLys ER was not influenced by dietary dLys level. In the first phase (0-21days), the diets consisted of 12.03 MJ/kg AMEn (low density) at 1.021, 1.139 and 1.257% dLys giving dLys: AMEn ratios 0.85, 0.95 and 1.04, and 13.23 MJ/kg (high density) at 1.125, 1.255 and 1.385% giving dLys: AMEn ratios 0.92, 1.03 and 1.13. In the second phase (22-42days), the diets consisted of 12.64 (low density) MJ/kg AMEn at 0.922, 1.045 and 1.167% dLys giving dLys; AMEn ratios 0.73, 0.83 and 0.92; and 13.89 MJ/kg (high density) at 1.011, 1.146 and 1.280% dLys giving dLys: AMEn ratios 0.73, 0.83 and 0.92. There was an interaction ($P< 0.05$) between nutrient density and dLys: AMEn and the highest dLysER was observed in broilers fed high-density-high dLys and low-density-high dLys diets. At the same time, the lowest was recorded in broilers fed high-density low-dLys diets.

Nasr *et al.* (2011) also observed a significant difference in Lys ER with incremental changes in dLys (0.82, 0.89, 0.98 and 1.09%), AME was kept constant at 12.97 MJ/kg giving dLys: AME ratios 0.63, 0.69, 0.76 and 0.84. The dLys ER improved with the increase in dLys: AME ratio for the overall 22 to 42-day test period. Although Barekatain *et al.* (2021b) reported an improved utilisation in low AMEn density diets, AMEn concentration was changed in the second phase. It is possible that the higher levels of dLys and constant energy resulted in an imbalance. Although FI was not affected, it is possible that the imbalance could have reduced the efficiency of Lys utilization because there was not enough energy to synthesize protein and therefore there is less retention. The observed linear increase in dLys intake and decrease in dLysER suggest that increasing dLys intake beyond a specific level, about 1.10%, as illustrated in Figure 4.2, will negatively impact feed efficiency.

The increasing quadratic response of BWG to increasing dietary dLys suggests that adjusting the dLys: AMEn ratio in isoenergetic diets improves BWG until a maximum point beyond which it will exhibit diminishing gains. Body weight gain only differed ($P<0.05$) in week two (21-28 days) and the overall period (14-35 days), particularly, treatment 1 (lowest) and treatments 6 and 8 (highest). The differences manifested after 14 days, and this observation could be because, in the first week (14-21 days), the broilers were still getting used to the different dietary treatments, and towards depletion the broilers were approaching their maximum potential (growth deceleration). Nasr and Kheiri (2011) assessed the effect of varying dLys levels at constant AME concentrations. They found significant differences in BWG, and broilers fed high dLys diets recorded higher values than those provided lower dLys levels. The diets contained 12.72 MJ/kg AME (0-21 days) and 13.26 MJ/kg AME, and in both phases, dLys were formulated to contain 90, 100,110 and 120% of NRC (1994) broiler Lys specifications. Barekatain *et al.* (2021b) also evaluated the effect of increasing the dLys: AME ratio while feeding diets comprising 12.03 (low) and 13.26 (high), and 12.65 MJ/kg (low) and 13.87 MJ/kg (high) for the starter (0-21 days) and grower-finisher phases (22-42 days), respectively. Even though chickens that were fed energy-dense diets performed better overall ($P<0.05$), increasing the dLys: AME ratio significantly improved BWG irrespective of dietary energy density. Therefore, incremental adjustments in the dLys: AMEn ratio may be expected to improve broiler performance.

The decreasing linear response in FCR values indicates that increasing dLys improves overall broiler feed utilisation. Feed conversion ratio improved with incremental changes in dLys ($P<0.05$), except in the first week (14-21 days) after the commencement of the experiment. This observation could be because the broilers were still adjusting to the dietary treatments. Zarghi *et al.* (2020) obtained similar results when dLys increased (0.88, 0.94, 1.00, 1.06 and 1.12%) while dietary AME was maintained at 13.28 MJ/kg; Cobb 500 broilers were fed the different diets from 22 to 38 days of age. Hirai *et al.* (2022) also observed a significant improvement in FCR when dLys increased (0.88, 1.28 and 1.44%) while dietary ME was maintained at 12.77 MJ/kg; Cobb MV x Cobb 500 broilers were fed varying diets from 14 to 41 days of age. On the contrary, Nasr and Kheiri (2011) observed no difference in FCR with incremental changes in dLys (0.79 to 1.08%, 90 to 120% of NRC recommended levels) while maintaining dietary AME at about 13.28 MJ/kg ($P>0.05$); the diets were fed to Arian broiler chickens from 22 to 42 days of age. So, even though it may be expected for FCR to improve with higher nutrient densities, other factors, such as genetic strain, may also influence broiler responses (Zhang *et al.*, 2020).

4.6 Conclusion

There were no significant differences in FI due to dietary treatments. Traditionally, dietary AMEn levels increase as broilers age to meet their requirements for maintenance and production. So, it may be expected that AMEn will exhibit less effect on FI if a constant AMEn level is used for growing and finishing broilers compared to other factors, such as gut capacity. Also, broilers may be expected to keep consuming feed regardless of dLys concentration in such diets. The dLys ER and EER could be valuable indicators of feed efficiency, which could be incorporated into diet optimization models. In conclusion, by optimizing dLys levels in isoenergetic diets, broiler diet formulators and producers can effectively enhance growth performance and feed efficiency.

Chapter 5

General discussion and conclusion

For the past decades, the broiler industry has achieved a significant amount of genetic improvement, resulting in higher growth rates (Marcu *et al.*, 2013; Haque *et al.*, 2020). However, the industry faces multiple challenges that require new and innovative production practices to ensure sustainability (Al-Nasser *et al.*, 2015). Increasing feed costs is one of the major issues requiring producers to seek cost-effective alternatives. To achieve optimal growth, broilers require a well-balanced diet tailored to their specific energy and nutrient needs. Dietary energy and protein are the most significant cost factors in broiler diets. Literature indicates that as a strategy to mitigate the issue of high feed cost, broilers can be fed lower nutrient-density diets; however, some studies have also reported reduced growth performance with such diets. Therefore, broiler performance should be prioritised while considering the practical consequences of diet composition (Hirai *et al.*, 2020). The primary aim of the present study was to evaluate broiler responses to varying AMEn and nutrient (dLys) levels.

Feed intake and live performance of Cobb 500 broilers fed on incremental dietary AMEn concentrations while maintaining a constant dLys: AMEn ratio (0.97) were evaluated in Chapter 3. It was hypothesized that incremental changes in dietary AMEn concentration at a constant dLys: AMEn ratio would reduce FI and improve growth performance. Feed intake decreased linearly with gradual changes in AMEn, which could be explained based on the idea that broilers primarily consume feed to satisfy their energy requirements (Scanes, 2022). Dietary AMEn intake also increased linearly, while EER was not significantly affected. The observed response is associated with dietary AMEn levels and suggests that even though birds may attempt to consume more feed to satisfy their daily needs, factors such as gut capacity will also limit AMEn intake. No significant differences were observed for overall BWG. Increasing AMEn while maintaining the dLys: AMEn ratio improved FCR. As a result, the hypothesis is partially accepted because FI decreased and FCR improved; however, there was no significant improvement in BWG with incremental changes in AMEn. In conclusion, broilers are able to maintain optimal growth when the ideal dietary AMEn, and nutrient balance is maintained when nutritional adjustments are made.

In Chapter 4, the hypothesis tested was that increasing dLys (manipulating the dLys: AMEn ratio) would significantly impact FI and enhance growth performance. Overall, FI was not affected by the incremental changes in dietary dLys. There were no significant differences in AMEn intake, while EER, dLys intake and dLysER differed significantly between dietary treatments. Digestible Lys intake increased while dLysER decreased as dLys inclusion level increased, resulting in a point of intersection that could indicate the optimal point for feed efficiency. Overall, BWG exhibited an increasing quadratic response, suggesting that beyond an optimal point, it would decrease with increasing dLys levels. Overall FCR also differed between dietary treatments and exhibited a decreasing linear response. Partial acceptance of the hypothesis suggested that there is a potential for feeding a constant AMEn concentration to grow and finish broilers when an optimal dLys: AMEn ratio is determined.

Although energy and nutrient-dense diets result in better broiler growth rates, they are not always economically feasible. Martins *et al.* (2016) indicated that FI, FCR, breast yield, and production efficiency index improved as dietary energy and nutrient density increased. However, higher nutritional and energy levels led to worse economic outcomes overall. In both experiments, feed cost increased with incremental changes in dietary AMEn and nutrient (dLys) density, see Appendix 1. In experiment 1, diet 1 had the lowest average feed cost at about R5.58/kg, while diet eight cost about R 7.26/kg. Since there were no differences in overall BWG, it would make financial sense not to choose diet eight to grow and finish the broilers. For example, the average BWG for diet 6 was 2.282kg with a feed cost of R6.70 while the average BWG for diet 8 was 2.254kg; therefore, it would cost about R1.07 less while producing about 28 grams more if diet 6 is chosen over diet 8. In experiment 2, however, BWG was optimised at a higher dLys; AMEn ratio, so the high nutrient specification diet makes sense. Still, the high nutrient specification diet in experiment 1 cost about R7.26 compared to R6.57 in experiment 2, suggesting that optimising the dLys: AMEn ratio and selecting one optimal AMEn concentration instead of multi-energy diets for growing and finishing broilers could benefit least-cost formulation. Therefore, it is recommended that before decisions on which dietary energy and nutrient density are suitable, economic implications must also be considered.

Reviewed literature (section 2.6.2) points that dietary AME and amino acids (i.e. dLys) significantly influence other broiler performance measure such as carcass yield, abdominal fat pad and carcass chemical composition. Therefore, future studies could investigate the effect of the different dietary treatments which were used during the present trials on carcass measures for potential market-specific requirements and demands. Research also shows that bird sex may influence broiler responses to varying dietary ME density and CP/AA levels, potentially impacting production parameters such as growth performance (Shahin & Abd El Azeem, 2006; Mutibvu *et al.*, 2020). So, because the birds used during the course of the current trials were as hatched (un-sexed), testing similar diets on sexed birds could benefit producers who favour sexed flocks. Broiler meat producers depend on the knowledge and expertise of researchers and diet formulators. Thus, optimising broiler responses by identifying the ideal energy and nutrient balance will allow for better and more robust feeding strategies and improve broiler performance.

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Appendix 1: Broiler feed cost per tonne

Dietary Treatment	Experiment 1	Experiment 2
1	R5567.58	R5438.11
2	R5732.43	R5560.19
3	R5989.23	R5704.28
4	R6217.48	R5879.89
5	R6431.88	R6061.53
6	R6700.12	R6230.27
7	R7006.62	R6398.42
8	R7264.31	R6573.25