UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

ASSESSMENT OF DIFFERENT FEED FORMULATIONS WHILE REDUCING DIETARY CRUDE PROTEIN ON PERFORMANCE OF BROILER CHICKENS

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IMPACTO POTENCIAL DESTA PESQUISA

O rápido crescimento dos frangos de corte justifica suas altas exigências de proteína. No entanto, a atender o Objetivo de Desenvolvimento Sustentável (ODS) 2 (fome zero), sem diminuir os objetivos 12 (consumo e produção responsáveis), 13 (ação climática) e 15 (vida na terra), é necesário encontrar formas sustentáveis de produzir carne de frango sem impactar negativamente o meio ambiente nem reduzir sua produtividade. Uma possibilidade para reduzir a excreção de nitrogênio é rduzir a inclusão de proteína bruta nas dietas e incluir aminoácidos cristalinos. A redução da excreção de nitrogênio no meio ambiente é importante para diminuir a incidencia de eutrofização e formação de óxido nitroso. Além disso, os ingredientes disponíveis para rações, especialmente cereais, diferem em diferentes regiões. Este estudo tem como objetivo comparar o efeito de diferentes combinações de ingredientes principais (trigo-milho e sorgo-trigo) ao milho, reduzindo a proteína da dieta em 2% (20 g/kg) e 4% (40 g/kg) em frangos de corte. Talvez esta pesquisa possa encontrar uma alternativa para as dietas comprovadas de milho e soja enquanto reduz a proteína da dieta e também recomendar o nível em que a proteína pode ser reduzida nas dietas de frangos de corte.

A indústria avícola tem sido bastante lenta na adoção do sistema de energia líquida, em comparação com suínos e ruminantes. No entanto, este estudo mostra que, ao formular dietas para frangos de corte com uma energia metabolizável aparente fixa, reduzindo a proteína da dieta, a energia líquida das dietas aumenta com a diminuição do nível de proteína. Talvez este estudo possa ajudar a destacar, de alguma forma, a importância do uso do sistema de energia líquida na formulação de rações para frangos.

POTENTIAL IMPACT OF THIS RESEARCH

The rapid growth of broiler chickens justifies their high protein requirements. However, the need for the world to meet the Sustainable Development Goal (SDG) 2 (zero hunger), while not diminishing goals 12 (responsible consumption and production), 13 (climate action), and 15 (life on land) necessitates the need to find sustainable ways to produce chicken meat without negatively impacting the environment nor reduce their productivity. One of the ways to achieve this is by reducing the dietary protein and supplement the diets with the crystalline amino acids, as this will reduce the nitrogen they excrete into the environment, thus reducing their contribution to eutrophication, and formation of nitrous oxide. Also, the available feed ingredients, especially cereals, in different regions differ. This study aims to compare the effect of different major ingredient combinations (wheat-corn and sorghum-wheat) to corn while reducing the dietary protein by 2% (20 g/kg) and 4% (40 g/kg) in broiler chickens. Perhaps this research can find an alternative to the proven corn-soy diets while reducing dietary protein, and also recommend the level to which protein can be reduced in broiler diets.

The poultry industry has been quite slow in adopting the net energy system, compared to swine and ruminants. However, this study shows that when formulating broiler diets with a fixed apparent metabolizable energy while reducing the dietary protein, the net energy of the diets increases with decreasing protein level. Perhaps, this study may help highlight, to a little extent, the importance of using the net energy system in chicken feed formulation.

UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal

CERTIFICADO DE APROVAÇÃO

TÍTULO DA DISSERTAÇÃO: ASSESSMENT OF DIFFERENT FEED FORMULATIONS WHILE REDUCING DIETARY CRUDE PROTEIN ON PERFORMANCE OF BROILER CHICKENS

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Jaboticabal, 10 de maio de 2023

AUTHOR'S CURRICULUM DATA

Damilola Uthman Kareem, born on March 26, 1992, in Ibadan, Oyo State, Nigeria. He was admitted to the Department of Animal Nutrition, Federal University of Abeokuta, Nigeria, to study Animal Nutrition, in September 2011. In February 2017, he graduated with a First class (Hons.) and obtained the title of Bachelor of Agriculture (Animal Nutrition). He went for the Nigerian National Youth Service in 2017 and concluded service in 2018. He commenced a master's program (Monogastric Animal Nutrition) in the Department of Animal Nutrition, Federal University of Abeokuta, Nigeria, in 2018, and graduated in 2021. He was employed by the Federal University of Agriculture Abeokuta, Nigeria, in August 2019, as a Graduate Assistant. In 2021, he was admitted for a master's degree in animal science at the Universidade Estadual Paulista – "Júlio de Mesquita Filho" (UNESP) – Faculty of Agricultural and Veterinary Sciences, Jaboticabal Campus, sponsored by a collaboration of the Tertiary Education trust Fund (TetFund) and Agricultural Research and Innovation Fellowship for Africa (ARIFA), under the supervision of Profª. Drª. Nilva Kazue Sakomura and defended his dissertation on 10 May 2023.

Invictus (A poem)

Out of the night that covers me,
Black as the pit from pole to pole,
I thank whatever gods may be
For my unconquerable soul.

In the fell clutch of circumstance
I have not winced nor cried aloud.
Under the bludgeonings of chance
My head is bloody, but unbowed.

Beyond this place of wrath and tears
Looms but the horror of the shade,
And yet the menace of the years
Finds and shall find me unafraid.

It matters not how strait the gate,
How charged with punishments the scroll,
I am the master of my fate,
I am the captain of my soul.

(William Ernest Henley, 1875)

Dedication

To the two wonderful fairies in my life; my beautiful wife, Toyyibah, and my precious daughter, Zara, for their perseverance.

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My profound gratitude to God for the gift of life.

Completing a dissertation is not a solitary endeavor; rather, it represents the culmination of the support, guidance, and encouragement provided by numerous individuals who have contributed to the journey.

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I would be an ingrate, if I forget to acknowledge the love and support of *minha família brasileira*; Marcia, Alcimar, José and his family. I will miss the outings and *churrascos*. I hope I can repay your kindness in the near future.

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I offer my regards to all those who supported me in any respect, but who I may have inadvertently omitted, perhaps due to oversight or limited paper space. Not mentioning your names does not mean I forgot you. I am so grateful for your deeds.

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"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Avaliação de diferentes estratégias de formulação de alimentos para frangos de corte sobre redução do teor de proteína bruta"**, protocolo nº 639/22, sob a responsabilidade da Profª Drª Nilva Kazue Sakomura, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 16 de fevereiro de 2022.

Vigência do Projeto	20/03/2022 a 20/12/2022
Espécie / Linhagem	*Gallus gallus* – Cobb500
Nº de animais	2400
Peso / Idade	42g / 1 dia
Sexo	Macho
Origem	Incubatório Pluma agroavícola – Descalvado - SP

Jaboticabal, 20 de fevereiro de 2022.

Fabiana Pilarski
Profª Drª Fabiana Pilarski
Coordenadora – CEUA

AVALIAÇÃO DE DIFERENTES ESTRATÉGIAS DE FORMULAÇÃO DE DIETAS COM REDUÇÃO DE PROTEÍNA BRUTA PARA FRANGOS DE CORTE

RESUMO - A composição nutricional das dietas das aves pode variar dependendo da disponibilidade de ingredientes, impactando potencialmente o desempenho dos frangos de corte, especialmente quando alimentados com dietas com proteína bruta (PB) reduzida. Um total de 2.304 pintos de corte Ross 308, machos, com um dia de idade, foram arranjados em esquema fatorial 3 x 3. As aves foram alimentadas com ração inicial padrão de 0 a 7 dias. No d 8, elas foram distribuídas aleatoriamente em nove tratamentos de oito repetições com 32 aves cada. As dietas experimentais foram elaboradas a partir de três combinações de ingredientes (milho; trigo-milho; sorgo-trigo) com três níveis de PB (Controle; Moderado [controle menos 20 g/kg PB]; forte redução [controle menos 40 g/kg PB]) na fase I (230, 210 e 190 g/kg PB), na fase II (215, 195 e 175 g/kg PB) e na fase final (205, 185 e 165 g/kg PB). A conversão alimentar (FCR) foi semelhante e melhor para as aves alimentadas com proteína moderada durante a fase inicial de crescimento (8 - 28 d), mas piorou à medida que os níveis de PB diminuíram durante a fase de terminação. Independentemente da dieta, a ingestão de proteína e gordura diminuiu à medida que os níveis de PB na dieta diminuíram, enquanto a deposição de gordura e a eficiência da deposição de proteína e gordura aumentaram. As aves excretaram menos nitrogênio à medida que o nível de PB diminuiu. As dietas à base de milho apresentaram os maiores pesos de carcaça e rendimento de peito para os três níveis de PB, enquanto trigo-milho e sorgo-trigo apresentaram valores semelhantes, apenas nos níveis de PB controle e moderado. A digestibilidade da PB foi maior para dietas à base de milho e trigo-milho e menor para as rações contendo os níveis de PB controle e moderado. As dietas à base de milho tiveram a maior digestibilidade energética, mas a energia digestível das dietas à base de milho e sorgo-trigo foi semelhante. A redução de 40 g proteína bruta por kg de ração resultou em aumento na digestibilidade dos aminoácidos, quando comparado as rações controle e moderada. A ração contendo milho apresentou a melhor (maior) digestibilidade dos aminoácidos em comparação com trigo-milho e sorgo-trigo. O estudo revelou que as aves alimentadas com dietas de trigo-milho ou sorgo-trigo tiveram peso corporal menor, mas semelhante. No entanto, os frangos alimentados com dieta à base de milho registraram a melhor conversão alimentar. Portanto, a redução de 20 g/kg de PB nas dietas de frangos de corte permite a redução da excreção de nitrogênio sem impactar negativamente no rendimento de carcaça, rendimento de peito e deposição de proteína dos frangos de corte.

Palavras-chave: aminoácidos, deposição de gordura, deposição de proteína, excreção de nitrogênio, proteína bruta reduzida

ASSESSMENT OF DIFFERENT FEED FORMULATIONS WHILE REDUCING DIETARY CRUDE PROTEIN ON PERFORMANCE OF BROILER CHICKENS

ABSTRACT - The nutrient composition of poultry diets can vary depending on ingredient availability, potentially impacting broiler performance, especially when fed as reduced crude protein (CP) diets. A total of 2,304 male, one-day-old Ross 308 broiler chicks were arranged in a 3 x 3 factorial. The birds were fed a standard starter diet from 0 to 7 d. On d 8, they were randomly allotted to nine dietary treatments of eight replicates with 32 birds each. The experimental diets were of three ingredient combinations (Corn; wheat-corn; sorghum-wheat) with three CP levels (Control CP; Moderate CP reduction [control minus 20 g/kg CP]; Strong CP reduction [control minus 40 g/kg CP]) at the grower I (230, 210, and 190 g/kg CP), grower II (215, 195, and 175 g/kg CP), and finisher (205, 185, and 165 g/kg CP) phases. The birds' feed conversion ratio (FCR) was similar and better for those fed control and moderately reduced protein during the early growth stage (8 - 28 d) but worsened as CP levels reduced during the finisher phase. Regardless of the diet, protein and fat intake decreased as dietary CP levels decreased, while fat deposition, and ratio of protein and fat deposition to intake increased. Birds excreted less nitrogen as the CP level decreased Birds excreted less nitrogen as the CP level decreased. Corn-based diets had the highest carcass weight and breast yield for all three CP levels, while wheat-corn and sorghum-wheat had similar values but only at control and moderately reduced CP levels. CP digestibility was higher for corn-based and wheat-corn diets and lower for control and moderately reduced CP levels. Corn-based diets had the highest energy digestibility, but the digestible energy of corn-based and sorghum-wheat diets was similar. While the amino acid digestibility is similar and lower for control and moderately reduced CP levels, corn had the best (highest) digestibility compared to wheat-corn and sorghum-wheat. The study revealed that birds fed with wheat-corn or sorghum-wheat diets had lower but similar BWG, and irrespective of the dietary composition, there is no difference in BWG up to 20 g/kg CP reduction. However, the broilers fed a corn-based diet recorded the lowest (best) FCR. Therefore, the reduction of 20 g/kg of CP in broiler chickens' diets allows the reduction of nitrogen excretion without negatively impacting the broiler chickens' carcass yield, breast yield, and protein deposition.

Keywords: Amino acid, fat deposition, nitrogen excretion, protein deposition, reduced crude protein

LIST OF ABBREVIATIONS

ADP – Adenosine diphosphate

AIDC – Apparent ileal digestibility coefficient

AME_n - Apparent metabolizable energy corrected for zero nitrogen retention

AN_e – Apparent nitrogen excretion

AN_eC – Apparent nitrogen excretion coefficient

ANOVA – Analysis of variance

ATP – Adenosine triphosphate

BW – Body weight

BWG – Body weight gain

CO_2 – Carbon (iv) oxide

CP – Crude protein

DCL - Digestible crude lipid

DCP - Digestible crude protein

DE – Digestible energy

DM – Dry matter

ED – Energy digestibility

EE – Effective energy

FCR – Feed conversion ratio

Fd – Fat deposition

FI – Feed intake

FOM - Feed organic matter

GE – Gross energy

Gln – Glutamine

Glu – Glutamate

kF – Ratio of fat deposition to intake

kP – Ratio of protein deposition to intake

Mg – Magnesium

N – Nitrogen

Na – Sodium

NE – Net energy

NH_3 – Ammonia

NH_4 – Ammonium

N_i – Nitrogen intake

N_R – Nitrogen retained

Pd – protein deposition

P_i – Inorganic phosphorus

SID – Standardized ileal digestibility

TiO_2 – Titanium dioxide

w_d – Faecal organic matter

w_u – Unrinary nitrogen

z – Proportion of lipid retained

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CHAPTER 1 - General considerations

1.1 Introduction

Livestock production significantly impacts the environment, as evidenced by various studies (Steinfeld et al., 2006; de Vries and de Boer, 2010; Leip et al., 2015; Meier et al., 2015). However, the intensification of pig and poultry production, driven by efficiency, has led to environmental problems in some parts of the world (Ferket et al., 2002; Mallin et al., 2003). Therefore, it is essential to develop diets with reduced crude protein (CP) for broiler chickens, as accepting such diets would bring several advantages, including reduced environmental waste.

According to Chrystal et al. (2020a), a reduced-crude protein (CP) diet has 2% to 3% less CP than a conventional diet, achieved through a decrease in soybean meal and an increase in feed grain content, along with additional non-bound amino acids to meet the animal's protein and amino acid requirements. Greenhalgh et al. (2020) opined that reducing crude protein in a broiler's diet is a promising strategy to obtain sustainability in chicken-meat production as they reduce nitrogen (N) and ammonia (NH_3) emissions, which are increasingly important in today's world of environmental sustainability. Problems arising from litter quality have become a welfare concern, but dietary CP reduction has been shown to enhance litter quality and reduce footpad dermatitis and other lesions (van Harn et al., 2019). Furthermore, reduced-CP diets can decrease undigested protein flow into the large intestine, thus preventing the proliferation of potential pathogens and consequently reducing the need for in-feed antibiotics, as undigested protein and amino acids have been shown to influence the populations of *Clostridium perfringens*, the causative organism of necrotic enteritis (Greenhalgh et al., 2020; Wilke et al., 2005).

The world human population has been estimated to reach more than 9 billion by 2050, and studies show that this could increase the need for agricultural production by 50% to meet food demand (Alexandratos, 2012; Lombardi et al., 2021), thereby also increase the global demand for animal protein (Parrini et al., 2023). Broiler chicken production consumes a significant portion of the global soybean meal supply, with 44% used in the US and 32% in Europe (Dei, 2011). Global soybean production is expected to increase by 10.4% from 368.5 million tons in 2020 to 406.8 million tons in 2027 (OECD, 2018), with a corresponding 8.73% increase in soybean prices, especially in Europe. Due to the limited cultivated area available in Europe which makes soybean cultivation more difficult, the ban of animal products in poultry feed, increasing cost of soybeans as a result of increased cost of transportation (Parrini et al., 2023), and the need to reduced carbon footprint (de Boer et al., 2014), European countries have been trying hard to find suitable replacements for soybean meal as evidenced in the report of de Boer et al. (2014). However, despite the availability of several protein alternatives, soybean meal remains popular due to its high CP content of 44-48% (Rostagno et al., 2017), and well-balanced amino acid profile compared to other protein sources derived from oilseeds and cereals (Beski et al., 2015). Although it has been reported that available alternatives to soybean meal which includes canola meal (Ghazalah et al., 2021), pea, lupin seed, fava bean (Parrini et al., 2023) etc, as well as animal protein, where permissible, can provide broiler starter diets with adequate CP levels of around 22% (Greenhalgh et al., 2020). However, to meet the amino acid needs of modern broiler chickens, feeds must be supplemented with commercially available amino acids, such as methionine, lysine, and threonine, which has been a common practice for many years (Kidd et al., 2013).

Dietary CP reductions have been reported to generate tangible fluctuations in intestinal uptake of amino acids which have the potential to prompt amino acid imbalances (Liu et al., 2021). Many researchers (Chrystal et al., 2020a; Selle et al., 2020; Liu et al., 2021) have suggested that amino acid imbalances are more likely to occur in reduced-protein diets. However, Liu et al. (2021) opined that these fluctuations are almost certainly compounded by further variations in the post-enteral availability of amino acids, since the availability of amino acids is not solely determined by their intestinal uptakes, after entering the intestines. This is because, instead of directly entering the bloodstream, a significant quantity of amino acids may enter either the catabolic or anabolic pathways within the enterocytes, which generates the energy that fuels digestive processes or the synthesis of various proteins, such as mucin and other metabolites (Reeds et al., 1999; 2000).

Corn has been reported to be the best energy source when feeding a reduced CP diet to broiler chickens (Chrystal et al., 2021) despite different ingredient combinations including wheat (Hilliar et al., 2019), wheat-sorghum (Hilliar et al., 2020), and corn (Chrystal et al., 2021). Selle et al. (2021) suggested that wheat-based diets exacerbate amino acid imbalances compared to corn-based diets when dietary protein is reduced, because they found a higher proportion of uric acid nitrogen (N) to total N in excreta of birds offered reduced CP (165 g/kg CP) wheat-based diets (38%) compared to those fed maize-based diets (27.4%), resulting in deamination of surplus amino acids, hence ammonia accumulation. As a result, they concluded that ammonia overload may be partially responsible for the grossly inferior growth performance of birds offered reduced CP, wheat-based diets (and perhaps of other sources than corn).

The effects of reducing dietary CP in broiler chickens have been well reported including but not limited to digestibility (Chrystal et al., 2021, 2020b; Liu and Selle, 2017); growth performance (Chrystal et al., 2021, 2020c; Selle et al., 2021); starch and protein digestion dynamics (Chrystal et al., 2021; Greenhalgh et al., 2022; Liu and Selle, 2017; Selle et al., 2021; Selle and Liu, 2019); nitrogen and uric acid concentration (Selle et al., 2021); gut microbiome (Ravangard et al., 2017); litter quality (Alfonso-Avila et al., 2022; van Harn et al., 2019) etc, using corn and/or wheat as energy sources, very few have explained the impacts of reduced CP in broilers from the perspective of protein and/or fat deposition (Bregendahl et al., 2002; Freitas et al., 2023; Indarsih and Pym, 2009; Namroud et al., 2008). Although, according to Liu et al. (2021), moderate reductions of CP up to 3 % are quite feasible, but more tangible reductions usually compromise feed conversion ratios (FCR) with associated increases in fat deposition which may be monitored by relative fat-pad weights, and perhaps by the comparative slaughter method described by Sakomura and Rostagno (2016).

In this study, it was hypothesized that broiler chickens' response (nutrient digestibility, protein deposition, lipid deposition, growth performance, carcass yields) of broiler chickens due to dietary CP intake will slightly differ according to the feed's main ingredients (more specifically corn-soy, wheat-corn-soy, or sorghum-wheat-soy-based feeds). This study thereby aims to evaluate different diet formulations while reducing dietary crude proteins on the performance of broiler chickens.

1.2 Literature review

1.2.1 Broiler chicken production: Merits and challenges

Chicken meat, according to Mottet and Tempio (2017), is the fastest rising source of protein for human consumption, which is environmentally beneficial because chicken meat production produces fewer 'greenhouse gases' or carbon dioxide (CO_2) equivalents, than other meat protein sources (Selle et al., 2020). One kg of chicken meat was reported by Fiala (2008) to produce 1.1 kg CO_2 equivalents, which is considerably less than pig (3.8 kg CO_2 equivalents) or beef (14.8 kg CO_2 equivalents). However, the diets of broiler chickens may contain more than 200 g/kg (20 %) to 230 g/kg (23 %) of protein in its diet composition, which is higher than that required by other animals, with the majority of the protein coming from soybean meal. Given a conservative 250 g/kg dietary inclusion of soybean meal and a 2.25:1 feed-to-carcass weight conversion, Selle et al. (2021) deduced that 1 kg of chicken meat will require an input of 560 g soybean meal. In another study, Macelline et al. (2021) cited Tudorache et al. (2015), who stated that Ross 308 broiler chickens reach a live weight of 2.918 kg at 42 days post-hatch and a carcass weight of 2.151 kg after processing, equating to 376 g of carcass protein, given that the carcass contains 175 g/kg protein. In 42 days, these broiler chickens consumed 4.702 kg of feed, with dietary protein content decreasing from 230 to 183 g/kg and a weighted average of 201 g/kg protein. This corresponds to 945 g protein intake and 376 g output. To generate 1.00 kg of protein in a Ross 308 broiler chicken carcass or saleable final product, 2.51 kg of dietary protein is therefore required. They opined that other terrestrial food-producing animals do not require this much feed protein to carcass protein ratio of 2.51. Broiler chicken protein growth efficiency (33.3%) was assessed by Wu et al. (2014) to be much higher than that of pigs (23.3%) and feedlot cattle (12.1%). However, if reduced-CP diets could be designed such that a dietary reduction of 50 g/kg (5 %) CP would not impair growth performance, the dietary protein to carcass protein ratio of 2.51 would drop to 1.89, a significant 24.7 % improvement (Macelline et al., 2021).

1.2.2 Dietary sources of protein and amino acids in broiler nutrition

Macelline et al. (2020) reported diets are usually formulated on a least-cost basis to have diets that meet specified targets for a selected range of nutrients (digestible amino acids, energy density, etc) at the lowest cost possible. Numerous amino acid profiles to achieve the 'ideal protein ratio'. Li et al. (2011) reported the average amino acid compositions of six samples of relevant feedstuffs (soybean meal, fishmeal, meat and bone meal, corn, and sorghum), and Macelline et al. (2020) compared the results of Wu (2014) to Texas A&M optimal ratios for broiler chickens from 21 to 42 d post-hatch. They found that the linear relationships between amino acid ratios in feedstuffs with Texas A&M ratios showed that fish meal is the most closely aligned protein source, followed by meat and bone meal, soybean meal, sorghum, and then corn. According to Gorissen et al. (2018), animal protein sources are usually considered superior to vegetable proteins because their higher leucine, lysine, and methionine concentrations may have anabolic effects. However, the usage of animal proteins is restricted in non-ruminant diets as they are prohibited in the European Union and some other countries. Their prices may not be competitive due to demands from aquaculture and the pet food industry (Macelline et al., 2020), although the restriction on the use of animal products in Europe has been lifted slightly (European Commission, 2021). Their inclusions (meat and bone meal and other animal proteins) in diets for food-producing animals were banned in Europe, as a precaution, due to concerns over the possible transmission of zoonotic diseases such as *Bovine Spongiform Encephalopathy* ('Mad Cow' Disease) to humans, and animal welfare. Corn and wheat are the most used cereals as energy sources, in broiler diets globally (Macelline et al., 2020), while soybean meal is the most commonly used protein source.

1.2.3 Dependence of the poultry industry on soybean meal (SBM) and its impact

Soybean meal is not the only source of protein available for inclusion in broiler diets, but it is the dominant feedstuff in this respect (Selle et al., 2020), due to its high CP content of 44-48% (Rostagno et al., 2017) and well-balanced amino acid profile (Beski et al., 2015). USDA (2018) reported the global production of soybean to have doubled in the past 20 years, reaching 347 million tons in 2017/18, of which the majority (82%) was produced in the USA, Brazil, and Argentina. The global usage of soybean meal was 234 million tons in the same year, where China, the USA, and the European Union had the highest use (Selle et al., 2020).

Chicken-meat production absorbs a substantial proportions of soybean meal, as 44% in the USA, and 32% in Europe, was offered to broiler chickens (Dei, 2011). Global chicken-meat production has been predicted to double by 2050 (Kleyn, 2019) and has been projected to increase from 82 million tons in 2005/07 to 181 million tons in 2050 (Alexandratos, 2012). Selle et al. (2020) also estimated that there will be a 72% increase in chicken-meat production from 105.6 million tons in 2020 to 181.3 million tons in 2050, if previous projections of Alexandrtos (2012) and Kleyn (2019) are valid. This, perhaps a conservative projection, forecasts that an additional 76 million tons of chicken meat will be required in three decades to meet global demand, which might necessitate 55 million tons of whole soybeans or 43 million tons of soybean meal, accounting to over 20% of the current global supply of soybean meal (Selle et al., 2020). In fact, a more recent estimate by FAO (2022) reported the world poultry meat production in the year 2022 to be 138.8 million tons. This already exceeds the above estimates, making it more likely to have a higher production than the estimates above.

1.2.4 Digestion of protein and amino acids

Digestion and absorption of nutrients is an energy-demanding process, accounting for perhaps more than 20% of dietary energy (Cant et al., 1996). The protein digestion process begins in the proventriculus of poultry (Selle et al., 2020), where low pH caused by HCl secretions denatures proteins and allows pepsin to cleave them. The peptide end products of pepsin digestion trigger the release of cholecystokinin (CCK) and gastrin, which are thought to have a regulatory role in the entire protein digestion process (Krehbiel and Matthews, 2003).

Several pancreatic proteolytic enzymes (trypsin, chymotrypsin, peptidase, and elastase) are released into the duodenum to convert polypeptides to short peptide fragments (Selle et al., 2020). Aminopeptidase and dipeptidase are found in the apical membrane of enterocytes, which are converted into di- and tripeptides (or oligopeptides). The oligopeptide transporter, PepT-1, is responsible for the intestinal uptake of di- and tripeptides (Chen et al., 2005; Gilbert et al., 2010; Zwarycz and Wong, 2013). On the other hand, single, monomeric, or non-bound amino acids are absorbed through a variety of Na^+ - dependent and independent transport mechanisms with overlapping specificities and affinities (Miska and Fetterer, 2019). In response to Na^+ pump activity in the basolateral membrane of enterocytes, Na^+- dependent transporters are likely to be more prominent and co-absorb amino acids and sodium (Na). PepT-1, on the other hand, is not Na^+- dependent, and the distinction between monomeric and oligopeptide amino acids is significant (Selle et al., 2020). Krehbiel and Matthews (2003) asserted that 70 to 85% of amino acids are absorbed as oligopeptides, instead of monomeric amino acids.

Furthermore, oligopeptide uptake in the intestine is faster and more efficient than monomeric amino acids (Daniel, 2004; Gilbert, 2008). Although this may be true, Selle et al. (2020) asserted that polypeptides must first be digested into oligopeptides before the benefit becomes apparent. Therefore, they opined that reduced-CP diets might be disadvantaged in intestinal uptake of amino acids because they contain less intact protein, fewer oligopeptides, and more non-bound amino acids than standard-CP diets. Importantly, intestinal amino acid intake is likely to have a more significant impact on broiler growth than protein digestion (Croom et al., 1999).

1.2.5 Reduced crude protein in poultry nutrition

The concept of reduced protein diets is not new (Selle et al., 2020). Feed grade non-bound amino acids (methionine, lysine, and threonine) according to Kidd et al. (2012; 2013), have been routinely included in broiler diets for decades, and the availability of these amino acids has already allowed meaningful reductions in dietary CP and soybean meal inclusion levels in broiler diets (Pesti, 2009), although this may not seem as reducing CP in poultry diets. However, Selle et al. (2020) believed that the increasing commercial availability of the remaining proteinogenic amino acids should allow more tangible reductions in dietary CP and, in turn, greater declines in soybean meal inclusion levels.

An unpublished study quoted by Selle et al. (2020) reported that diets of reduced soybean up to 66% and supplemented with crystalline amino acids supported higher weight gain and feed intake in male broilers compared to those fed with diets of conventional CP. Although this may take a toll on the FCR, depending on the choice of ingredient used, this demonstrates that crystalline amino acids potentially hold as alternatives to soybean meal in chicken-meat production (Lemme et al., 2019). Nevertheless, some researchers (Belloir et al., 2017; Chrystal et al, 2020b; 2020c; 2020d) opined that CP reductions of this magnitude might compromise the efficiency of feed conversion with associated increases in fat deposition, which is said to probably stems, at least partially, from an insufficiently accurate identification of essential and non-essential amino acid requirements, or control protein ratios, in the context of reduced-CP diets.

1.2.6 Amino acid digestibility in broilers fed reduced-CP diets

Liu et al. (2021) noted that it would be wrong to assume that the reduction in crude protein of broiler chicken does not or have just a little impact on the amino acid digestibilty, due to the paucity of literature. Studies evidenced that amino acids' ileal and jejunal digestibility vary with CP level and amino acid (Awad et al., 2016; Hiliar et al., 2019; Hiliar et al., 2020; Chrystal et al., 2020c; 2020d). Liu et al. (2021) compiled some studies examining the impact of reduced CP on amino acid digestibilities. They reported that the mean percentage responses ranged from a decrease of 8.21% to an increase of 29.4% in the distal jejunal digestibility coefficients. In contrast, mean percentage responses ranged from a decrease of 8.36% to an increase of 7.43% in the distal ileum. Increases in amino acid digestibility coefficients with respect to reductions in dietary CP are partly attributed to the notional 100% digestibility of non-bound amino acids (Lemme et al., 2005). Amino acid digestibility increase or decrease with reduced dietary CP, based on the grain fed. Due to the competition between glucose and amino acids or among amino acids themselves for intestinal uptakes through co-absorption with sodium via their sodium-dependent or sodium-independent transport systems, amino acid digestibilities may be amplified or compromised by reductions in dietary CP (Liu et al., 2021).

1.2.7 Challenges of reduced crude protein diets

1.2.7.1 Amino acid imbalances

Dietary CP reductions have been suggested to cause amino acid imbalances (Huston and Scott, 1981), perhaps by generating tangible fluctuations in digestibilities and intestinal uptakes of amino acids which have the potential to prompt amino acid imbalances (Liu et al., 2021). Excessive plasma ammonia levels arising from imbalances and deamination of surplus amino acids can impede growth performance as a result of reduced feed intake, as there is a strong indication that elevated uric acid plasma concentrations are associated with reduced feed intake by the birds (Namroud et al., 2008; Ospina-Rojas et al., 2013; 2014; Liu et al., 2021).

1.2.7.2 Increased cost of deamination as a result of amino acid imbalance

Imbalances in amino acids have been reported to give rise to a surplus of catabolized amino acids (Bender, 2012), which could result in an increase in the costs of deamination and the possibility of excessive levels of ammonia being generated (Selle et al., 2020). Liu et al. (2021) opined that amino acid imbalances in birds offered reduced-CP diets could be a result of disparities in digestibility coefficients, different digestive kinetics of non-bound versus protein-bound amino acids, and the possibility that non-bound amino acids are more likely to be spared in their transition across the gut mucosa. The surplus amino acids undergo oxidative deamination in the liver, generating ammonia that requires detoxification (Stern and Mozdziak, 2019). Ammonia as reported by Liu et al. (2021), is detoxified by an energy-consuming, condensation reaction catalyzed by glutamine synthetase in which ammonia and glutamic acid are converted into glutamine as described by Minet et al. (1997) using the equation:

$$NH_4^+ + Glu + ATP + Mg^{2+} \Rightarrow Gln + ADP + Pi$$

Glutamine enters the uric acid cycle, thus allowing the N component of ammonia arising from deamination to be ultimately excreted as uric acid - N (Liu et al., 2021). There is an obligatory glycine input into the Krebs uric acid cycle where one mole of glycine is required for every uric acid excreted (Salway, 2018; van Milgen, 2021) and again, energy input is involved (Liu et al., 2021). These increased costs of deamination could reduce the energy to be utilized by the animal, thus rendering the calculated requirements of the animals inadequate.

1.2.7.3 Increased fat deposition

Due to the increased starch that ensue as a result of decreased dietary CP, lipid deposition in broiler chickens tend to increase. This results in the birds using the energy that would otherwise have been utilized for deamination and transamination of excess amino acids to metabolize the excess glucose to fat (Namroud et al., 2008). studiesVarious studies have observed these as increased abdominal fat recorded with decreased CP. Allameh and Toghyani (2019) reported that a reduction in the level of CP diets for broilers by 22 g/kg resulted in increased abdominal fat content. Hilliar et al. (2020) reported increased fat pad yield from 0.72% for control CP (20% CP) to 1.02% for least reduced CP (17% CP) in broiler chickens. Awad et al. (2015) also reported an increase in blood serum triglyceride level from 0.31 to at least 0.55 mmol/L, and abdominal fat content from 16.1 to at least 18.0 g/kg due to a reduction in the level of CP in broiler starter diets. Several other studies (Chrystal et al., 2021; Namroud et al., 2008) have observed the same trend.

1.2.8 Some factors to consider when feeding reduced CP diets

1.2.8.1 Starch and protein digestion dynamics

Selle and Liu (2019) reported that the fundamental premise of starch and protein digestive dynamics is that a controlled balance of glucose and amino acids is made available at sites of skeletal muscle protein synthesis to promote efficient growth. Thus, digestive dynamics involve the digestion of protein and starch in the gut lumen, absorption of glucose and amino acids along the small intestine and their transition across the gut mucosa into the portal circulation. Some researchers (Truong et al., 2017; Chrystal et al., 2020a) have suggested that greater focus should be placed on protein digestion rates for rapidly growing broiler chickens and their application in least-cost feed formulation. Also, Selle et al. (2015) reported that reducing dietary CP reduces soybean meal content, increases cereals (thereby starch), and consequently increases the content of non-bound amino acids in broiler diets. In light of this, Chrystal et al. (2020a) suggested that beyond the supply of adequate dietary amino acids, consideration of digestive dynamics of protein and starch is pivotal to the successful implementation of reduced CP diets where substantial quantities of non-bound amino acids are utilized.

Wu (2009) reported that non-bound amino acids do not require digestion, as they are immediately available for absorption in the small intestine and appear in the portal circulation more rapidly than protein-bound amino acids (Wu, 2009). They have inherently different digestive dynamics to protein-bound amino acids in broiler diets (Selle et al., 2015). Chrystal et al. (2020a) reported that rapidly digestible starch could flood the anterior small intestine with glucose to the extent that amino acids compete with glucose co-absorption with sodium and intestinal uptakes via their respective Na^+ -dependent transport systems. Hence, considering the existing studies (Liu and Selle, 2017; Moss et al., 2018a;b), Chrystal et al. (2020a) believed that slowly digestible starch might favor the absorption of amino acids into the portal circulation when reduced CP broiler diets with large amounts of supplemental, non-bound amino acids are fed. While starch typically increases with reductions in dietary CP, on the other hand, dietary lipid levels decrease (Selle et al., 2020). Liu et al. (2019) have found that elevated starch-to-lipid ratios reduced feed efficiency in broilers, but they observed that energy and amino acid densities had more pronounced impacts than starch-to-lipid ratios. This could be relevant in low-energy diets, where further fat reduction would not be feasible with least-cost formulations (Selle et al., 2020).

1.2.8.2 Glycine and serine (glycine equivalents)

Baker et al. (1968) have shown extensively the interrelationship between glycine and serine in poultry. Their combined dietary concentrations, according to Selle et al. (2020), are termed glycine equivalents and may be expressed according to the equation of Dean et al. (2006), where 1 mol of serine (105 g/mol) can be used to synthesize 1 mol of glycine (75 g/mol), serine would have an equivalence of 75/105 (0.7143) relative to glycine on a weight basis as simplified by van Milgen et al. (2021). The equation may be described as follows:

$$\text{Glycine equivalents (g/kg)} = \text{glycine (g/kg)} + [\text{serine (g/kg)} \times 0.7143]$$

The different routes of interconvertibility of glycine-serine and entry into the uric acid cycle, as described by van Milgen (2021), are presented in figure 1.

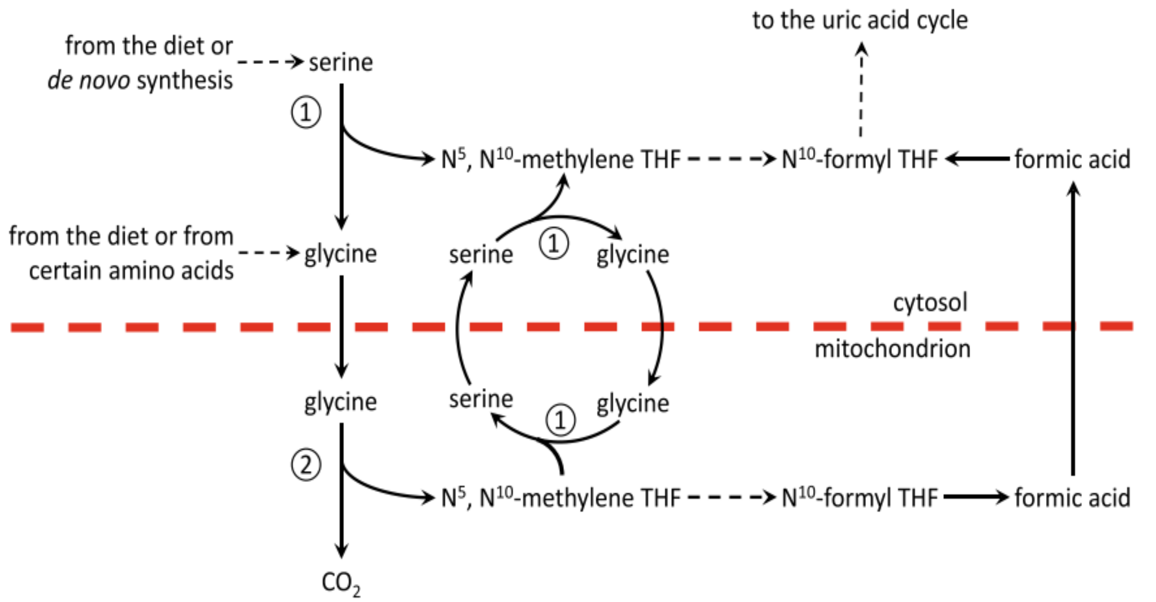

Fig. 1: Routes of interconvertibility of glycine-serine or vice-versa and their sites of action. ① = Serine hydroxymethyltransferase; ② = glycine cleavage system.

Source: van Milgen (2021)

Dean et al. (2006) and Baker (2009) reported that glycine supplementation in reduced-CP diets has improved growth performance of broiler chickens. Selle et al. (2020) opined that amino acid imbalances and consequent deamination of surplus amino acids in reduced-CP diets may amplify the need for dietary glycine. Its interconvertibility with serine (Selle et al, 2020, Liu et al., 2021; van Milgen, 2021) impacts the metabolism of methionine to cysteine (Selle et al., 2020), choline formation (Selle et al., 2020, van Milgen, 2021), and uric acid synthesis (Selle et al, 2020, Liu et al., 2021; van Milgen, 2021) elevates the importance of glycine equivalents in poultry, especially when feeding reduced CP diets. According to van Milgen (2021), the amount of glycine that the body retains in protein is higher than that of glycine consumed, resulting in a substantial amount of glycine required for uric acid. Additionally, while serine intake exceeds serine retention in body protein, the available serine is not enough to compensate for the glycine deficiency required for glycine retention in both body protein and uric acid during uric acid synthesis. This highlights the need to supplement glycine when there is a reduction in the required dietary CP.

It has been reported that, in theory, threonine can be metabolized to glycine (Baker et al., 1972), which is potentially significant (Liu et al., 2021). However, it was revealed by Liu et al. (2021) that elevations in threonine concentrations were quadratically related to declining glycine plasma levels (Chrystal et al., 2020b). They concluded that this outcome may indicate that threonine may not be metabolized to glycine in practice, which agrees with the opinion expressed by D'Mello (1973).

1.2.9 Net (effective) energy system and reduced CP: A consideration

Noblet et al. (2015) from the concept of Armsby and Fries (1915), defined the net energy of a diet as the metabolizable energy (ME) content minus heat increment (HI) associated with feed utilization (i.e., the energy cost of ingestion and HI related to metabolic utilization of ME) and the energy cost corresponding to an average level of physical activity per unit of diet ingested. Heat increment is defined by NRC (1981) as heat loss from fermentation, digestion and absorption, product formation, and waste formation and excretion. Emmans (1994) developed a new energy system called effective energy (EE), an improvement on the concept of net energy of Armsby and Fries (1915). Although the EE is not commonly used as the NE, because of the base concept, it is believed that EE is an advanced NE, so, in the concept of this review, NE and EE will be reported as NE, unless where specifically stated. In the current study, the NE values for the diets were calculated from the NE of each ingredient using the equation of Emmans (1994) expressed as:

$$\text{NE (kcal/kg)} = \text{AME}_n - (w_d \times \text{FOM}) - (0.16 \times w_u \times \text{DCP}) + (12 \times z \times \text{DCL})$$

Where; NE = Net energy; AME_n = Apparent metabolizable energy corrected to zero nitrogen retention; w_d = 3.80; FOM = Feed organic matter; w_u = 29.2; DCP = Digestible crude protein; z = 0.3; DCL = Digestible crude lipid

The concept of starch and protein dynamics (Chrystal et al., 2020a; 2021; Liu et al., 2021), starch : lipid ratio (Liu et al., 2019; Selle et al., 2020), energy density and utilization (Chrystal et al., 2021) had been used to explain the responses of broiler chickens to reduced CP diets. However, few studies have yet to attempt to explain responses in a reduced CP diet using the net energy approach. It was observed in this study that NE increases for all ingredient combinations as the crude protein reduces. This coincides with the fact that the lipid contents decreased with CP reduction. It has been reported by van der Klis and Jansman (2019) that the HI of fat is lowest compared to that of digested protein, starch, and NDF at 10, 42, 17, and 34%, respectively. The NE can be calculated by subtracting the HI from ME (Zuidhof, 2019; Sakomura and Rostagno, 2016). The reduced lipids in the diets with reduced CP resulted in a reduced HI, hence the increased NE. It is expected that there could be increased fat deposition in the birds fed reduced CP diets, as it has been shown in pigs (Batorek-Lukač et al., 2021; Moreira et al., 2022), and in laying hens (Barzegar et al., 2019) that fat deposition increased with increased NE. The fact that low-protein diets had been reported to increase abdominal fat deposition by Faria Filho (2003), and that Aletor et al. (2000) found that broilers fed diets with reduced crude protein had higher body fat deposition shows the correlation between reduced CP, increased NE, and increased fat deposition. While fat deposition increases as dietary protein reduces (Azeveido et al., 2021; Freitas et al., 2023), protein deposition has been shown to reduce as dietary protein reduces in (Freitas et al., 2023) broiler chickens. Although several attempts had been made to explain the impacts of reduced protein diets on broilers, it is believed that the NE system of energy partitioning may help explain more about what happens at the metabolic level.

1.3. References

Allameh, S., Toghyani, M., 2019. Effect of dietary valine supplementation to low protein diets on performance, intestinal morphology and immune responses in broiler chickens. **Livestock Science**, 229, 137–144. https://doi.org/10.1016/J.LIVSCI.2019.09.025

Aletor VA, Hamid II, Nieß E, Pfeffer E. (2000). Low-protein amino acid supplemented diets in broiler chickens: effects on performance, carcass characteristics, whole body composition and efficiencies of nutrient utilization. **Journal of the Science of Food and**

Agriculture, 80: 547-554. https://doi.org/10.1002/(SICI)1097-0010(200004)80:5<547::AID-JSFA531>3.0.CO;2-C

Alexandratos, N., Bruinsma, J. (2012). World Agriculture towards 2030/2050: The 2012 Revision. ESA Working paper No.12-03, Food and Agricultural, Organisation: Rome, Italy, pp. 1–154. https://www.fao.org/3/ap106e/ap106e.pdf

Armsby, H. P. & Fries, J. A. (I915). Net energy values of feeding stuffs for cattle. **Journal of'Agricultura1 Research**, 3, 435491. In: Emmans, G.C. (1994). Effective energy: a concept of energy utilization applied across species. **The British Journal of Nutrition**, 71: 801-821

Awad, E.A., Zulkifli, I., Soleimani, A.F., Loh, T.C., 2015. Individual non-essential amino acids fortification of a low-protein diet for broilers under the hot and humid tropical climate. **Poultry Science**, 94: 2772–2777. https://doi.org/10.3382/ps/pev258.

Awad, E.A., Zulkifli, I., Farjam, A.S., Chwen, L.T., Hossain, M.A., Aljoubori, A. (2016). Effect of low-protein diet, gender and age on the apparent ileal amino acid digestibilities in broiler chickens raised under hot-humid tropical condition. **Indian Journal of Animal Science**, 86(6): 696–701.

Azevedo, M.J., Reis, M.P., Gous, R.M., Dorigam, J.C.P., Leme, B.B., Sakomura, N.K. (2021) Response of broilers to dietary balanced protein. 1. Feed intake and growth. **Animal Production Science**, 61 (14):1425-1434. https://doi.org/10.1071/AN20655

Baker, D.H., Hill, T.M., Kleiss, A.J. (1972). Nutritional evidence concerning formation of glycine from threonine in the chick. **Journal of Animal Science**, 34(4): 582–6. https://doi.org/10.2527/jas1972.344582x

Baker, D.H. (2009). Advances in protein-amino acid nutrition in poultry. **Amino Acids**, 37: 29–41. https://doi.org/10.1007/s00726-008-0198-3

Baker, D.H., Sugahara, M., Scott, H.M. (1968). The glycine-serine interrelationship in chick nutrition. **Poultry Science**, 47: 1376–1377. https://doi.org/10.3382/ps.0471376

Barzegar, S., Wu, S.B., Noblet, J., Choct, M., Swick, R.A. (2019). Energy efficiency and net energy prediction of feed in laying hens. **Poultry Science**, 98: 5746–5758. http://dx.doi.org/10.3382/ps/pez362

Batorek-Lukač, N., Čandek-Potokar, M., Škrlep, M., Kubale, V., Labussière, E. (2021) Effect of Changes in Dietary Net Energy Concentration on Growth Performance, Fat Deposition, Skatole Production, and Intestinal Morphology in Immunocastrated Male Pigs. **Frontiers in Veterinary Science**, 8: 789776. https://doi.org/10.3389/fvets.2021.789776

Belloir, P., Méda, B., Lambert, W., Corrent, E., Juin, H., Lessire, M., Tesseraud, S. (2017). Reducing the CP content in broiler feeds: Impact on animal performance, meat quality and nitrogen utilization. **Animal**, 11: 1881–1889. https://doi.org/10.1017/S1751731117000660

Bender, D.A. (2012). The metabolism of “surplus” amino acids. **British Journal of Nutrition**, 108(S2): S113–21. https://doi.org/10.1017/S0007114512002292

Bregendahl, K., Sell, J.L., Zimmerman, D.R., (2002). Effect of Low-Protein Diets on Growth Performance and Body Composition of Broiler Chicks. **Poultry Science**, 81, 1156–1167. https://doi.org/https://doi.org/10.1093/ps/81.8.1156

Cant, J.P., McBride, B.W., Croom, W.J. (1996). The regulation of intestinal metabolism and its impact on whole animal energetics. **Journal of Animal Science**, 74: 2541–2553. https://doi.org/10.2527/1996.74102541x

Chen, H., Pan, Y.X., Wong, E.A., Webb, K.E. (2005). Dietary protein level and stage of development affect expression of an intestinal peptide transporter (cPepT1) in chicken. **The Journal of Nutrition**, 135: 193–198. https://doi.org/10.1093/jn/135.2.193

Chrystal, P.V., Shiva, G., Selle, P.H., Liu, S.Y. (2020a). Facilitating the acceptance of tangibly reduced-crude protein diets for chicken-meat production. **Animal Nutrition**, 6(3):247–257. https://doi.org/10.1016/j.aninu.2020.06.001

Chrystal, P.V., Moss, A.F., Khoddami, A., Naranjo, V.D., Selle, P.H., Liu, S.Y. (2020b). Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. **Poultry Science**, 99: 505–516. https://doi.org/10.3382/ps/pez573

Chrystal, P.V., Moss, A.F., Khoddami, A., Naranjo, V.D., Selle, P.H., Liu, S.Y. (2020c). Effects of reduced crude protein levels, dietary electrolyte balance and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein and amino acid metabolism. **Poultry Science**, 99: 1421–1431. https://doi.org/10.1016/j.psj.2019.10.060

Chrystal, P.V., Moss, A.F., Yin, D., Khoddami, A., Naranjo, V.D., Selle, P.H., Liu, S.Y. (2020d). Glycine equivalent and threonine inclusions in reduced-crude protein, maize-based diets impact on growth performance, fat deposition starch-protein digestive dynamics and amino acid metabolism in broiler chickens. **Animal Feed Science and Technology**, 261: 114387. https://doi.org/10.1016/j.psj.2019.10.060

Chrystal, P.V, Greenhalgh, S, McInerney, B.V, McQuade, L.R, Selle, P.H, Liu, S.Y. (2021). Maize-based diets are more conducive to crude protein reductions than wheat-based diets for broiler chickens. **Animal Feed Science and Technology**, 275: 114867. https://doi.org/10.1016/j.anifeedsci.2021.114867

Croom, W.J., Brake, J., Coles, B.A., Havenstein, G.B., Christensen, V.L., McBride, B.W., Peebles, E.D., Taylor, I.L. (1999). Is intestinal absorption capacity rate-limiting for performance in poultry? **Journal of Applied Poultry Research**, 8: 242–252. https://doi.org/10.1093/japr/8.2.242

D'Mello, J.P.F. (1973). Aspects of threonine and glycine metabolism in the chick (*Gallus domesticus*). **Annals of Nutrition and Metabolism**, 15(6): 357–63. https://doi.org/10.1093/japr/5.4.358

Daniel, H. (2004). Molecular and integrative physiology of intestinal peptide transport. **Annual Review of Physiology,** 66:361–84. https://doi.org/10.1146/annurev.physiol.66.032102.144149

de Boer, H.C., van Krimpen, M.M., Blonk, H., Tyszler, M. (2014). Replacement of soybean meal in compound feed by European protein sources: Effects on carbon footprint. Livestock Research Report 819, Wageningen UR Livestock Research. https://edepot.wur.nl/324258

de Vries, M., de Boer, I.J.M. (2010). Comparing environmental impacts for livestock products: A review of life cycle assessments. **Livestock Science,** 128, 1–11. https://doi.org/10.1016/j.livsci.2009.11.007

Dean, D.W., Bidner, T.D., Southern, L.L. (2006). Glycine supplementation to low protein, amino acid- supplemented diets supports optimal performance of broiler chicks. **Poultry Science**, 85: 288–296. https://doi.org/10.1093/ps/85.2.288

Dei, H.K. (2011). Soybean as a feed ingredient for livestock and poultry. In Recent, Trends for Enhancing the Diversity and Quality of Soybean, Products, Krezhova, D., Ed., InTech: Rijeka, Croatia, pp. 215–226.

Egli, D.B. (2008). Comparison of corn and soybean yields in the United States: Historical trends and future prospects. **Agronomy Journal**, 100 (Suppl. 3): S79–S88. https://doi.org/10.2134/AGRONJ2006.0286C

Emmans, G.C. (1994). Effective energy: a concept of energy utilization applied across species. **The British Journal of Nutrition**, 71: 801-821. https://doi.org/10.1079/bjn19940188

European Commission (2021). Authorisation to use certain proteins of animal origin to feed non-ruminant farmed animals. https://food.ec.europa.eu/system/files/2021-08/qa-animal-feed-auth-proteins_en_2.pdf. Accessed on April 11, 2023.

FAO (2022). Meat market review: Emerging trends and outlook. Food and Agriculture Organization of the United Nations, Rome. https://www.fao.org/3/cc3164en/cc3164en.pdf

Faria Filho, D.E. (2003). **Efeito de dietas com baixo teor protéico, formuladas usando o conceito de proteína control, para frangos de corte criados em temperaturas fria, termoneutra e quente**. Dissertação (Mestrado em Zootecnia) - Unesp, Jaboticabal. https://repositorio.unesp.br/handle/11449/99614

Ferket, P.R., van Heugten, E., van Kempen, T.A.T.G., Angel, R. (2002). Nutritional strategies to reduce environmental emissions from nonruminants. **Journal of Animal Science**, 80, E168–E182. https://doi.org/10.2527/animalsci2002.80E-Suppl_2E168x

Fiala, N. (2008). Meeting the demand: An estimation of potential future greenhouse gas emissions from meat production. **Ecological economics**, 67: 412–419. https://doi.org/10.1016/j.ecolecon.2007.12.021

Freitas, L.F.V., Dorigam, J.C.P., Reis, M.P., Horna, F., Fernandes, J.B.K., Sakomura, N.K. (2023). Eimeria maxima infection impacts the protein utilization of broiler chicks from 14 to 28 days of age, **Animal**, 100807. https://doi.org/10.1016/j.animal.2023.100807

Ghazalah, A. A., El-Kaiaty, A. M., Motawe, H. F. A., Radwan, A. S. (2021). Nutritional impact of canola meal on performance, blood constituents and immune response of broilers. **Journal of Agricultural Science**, 13 (1): 136 – 144. https://doi.org/10.5539/jas.v13n1p135

Gilbert, E.R., Li, H., Emmerson, D.A., Webb, K.E., Wong, E.A. (2010). Dietary protein composition influences abundance of peptide and amino acid transporter messenger ribonucleic acid in the small intestine of 2 lines of broiler chicks. **Poultry Science**, 89: 1663–1676. https://doi.org/10.3382/ps.2010-00801

Gilbert, E.R., Wong, E.A., Webb, K.E. (2008). Peptide absorption and utilization: Implications for animal nutrition and health. **Journal of Animal Science**, 86(9): 2135–2155. https://doi.org/10.2527/jas.2007-0826

Gorissen, S.H.M., Crombag, J.J.R., Senden, J.M.G., Waterval, W.A.H., Bierau, J., Verdijk, L.B., van Loon, L.C.J. (2018). Protein content and amino acid composition of commercially available plant-based protein isolates. **Amino Acids**, 50: 1685–1695. https://doi.org/10.1007/s00726-018-2640-5

Greenhalgh, S., Chrystal, P.V., Selle, P.H., Liu, S.Y. (2020). Reduced-crude protein diets in chicken-meat production: justification for an imperative. **World's Poultry Science Journal**, 76(3): 537-548. https://doi.org/10.1080/00439339.2020.1789024.

Hilliar, M., Huyen, N., Girish, C. K., Barekatain, R., Wu, S., & Swick, R. A. (2019). Supplementing glycine, serine, and threonine in low protein diets for meat type chickens. **Poultry science**, 98(12): 6857–6865. https://doi.org/10.3382/ps/pez435

Hilliar, M., Hargreave, G., Girish, C.K., Barekatain, R., Wu, S.B., Swick, R.A., 2020. Using crystalline amino acids to supplement broiler chicken requirements in reduced protein diets. **Poultry science**, 99(3), 1551–1563. https://doi.org/10.1016/J.PSJ.2019.12.005

Huston, R.L., Scott, H.M. (1968). Effect of varying the composition of a crystalline amino acid mixture on weight gain and pattern of free amino acids in chick tissue. **Federation Proceedings**, 27(5): 1204–9. https://doi.org/10.1590/S1516-635X2004000200001

Indarsih, B., Pym, R.A.E., 2009. The efficiency of protein utilization in different broiler strains. **Journal of the Indonesian Tropical Animal Agriculture**, 34, 167–173. https://doi.org/10.14710/jitaa.34.3.167-173

Kidd, M.T., Tillman, P.B. (2012). Feed additive mythbusters: How should we feed synthetic amino acids? **Proceedings of the Australian Poultry Science Symposium**, 23: 105–111.

Kidd, M.T., Tillman, P.B., Waldroup, P.W., Holder, W. (2013). Feed-grade amino acid use in the United, States: The synergetic inclusion history with linear programming. **Journal of Applied Poultry Research**, 22: 583–590. https://doi.org/10.3382/japr.2012-00690

Kleyn, R. (2019). Practical views on global meat chicken nutrition. **30th Proceedings of the Australian Poultry Science Symposium**, 30: 1–7. https://az659834.vo.msecnd.net/eventsairaueprod/production-usyd-public/1330f0d28dc54ef39afea020d58a4da9

Krehbiel, C.R., Matthews, J.C. (2003). Absorption of amino acids and peptides. In Amino Acids in Animal Nutrition, 2nd ed., D'Mello, J.P.F., Ed., CABI Publishing: Wallingford, UK, pp. 41–70.

Leip, A., Billen, G., Garnier, J., Grizzetti, B., Lassaletta, L., Reis, S., Simpson, D., Sutton, M.A., de Vries, W., Weiss, F. (2015). Impacts of European livestock production: Nitrogen, sulphur, phosphorus and greenhouse gas emissions, land-use, water eutrophication and biodiversity. **Environmental Research Letter**, 10. https://doi.org/10.1088/1748-9326/10/11/115004

Lemme A, Rostagno HS, Petri A, Albino LF. (2005). Standardised ileal digestibility of crystalline amino acids. **15th European Symposium on Poultry Nutrition**, 462–464. Balatonfured: World's Poultry Science Journal.

Lemme, A., Hiller, P., Klahsen, M., Taube, V., Stegemann, J., Simon, I. (2019). Reduction of dietary protein in broiler diets not only reduces n-emissions but is also accompanied by several further benefits. **Journal of Applied Poultry Research**, 28: 867–880. https://doi.org/10.3382/japr/pfz045

Li, X., Rezaei, R., Li, P., Wu, G. (2011). Composition of amino acids in feed ingredients for animal diets. **Amino Acids**, 40: 1159–1168. https://doi.org/10.1007/s00726-010-0740-y

Liu SY, Selle PH. (2017). Starch and protein digestive dynamics in low-protein diets supplemented with crystalline amino acids. **Animal Production Science**, 57: 2250e6.

Liu, S.Y., Macelline, S.P., Chrystal, P.V., Selle, P.H. (2021). Progress towards reduced-crude protein diets for broiler chickens and sustainable chicken-meat production. **Journal of Animal Science and Biotechnology,** 12: 1-13. https://doi.org/10.1186/s40104-021-00550-w

Liu, S.Y., Naranjo, V.D., Chrystal, P.V., Buyse, J., Selle, P.H. (2019). Pen-Behnken optimisation of growth performance, plasma metabolites and carcass traits as influenced by dietary energy, amino acid and starch to lipid ratios in broiler chickens. **PLoS ONE**, 14: e021387545. https://doi.org/10.1371/journal.pone.0213875

Lombardi, G., Parrini, S., Atzori, R., Stefani, G., Romano, D., Gastaldi, M., Liu, G. (2021). Sustainable agriculture, food security and diet diversity. The case study of Tuscany, Italy. **Ecological Modelling**, 458: 109702. https://doi.org/10.1016/j.ecolmodel.2021.109702

Macelline, S.P., Chrystal, P.V., Liu, S.Y., Selle, P.H. (2021). The Dynamic Conversion of Dietary Protein and Amino Acids into Chicken-Meat Protein. **Animals,** 11: 2288. https://doi.org/10.3390/ani11082288

Mallin, M.A., Cahoon, L.B. (2003). Industrialized animal production—A major source of nutrient and microbial pollution to aquatic ecosystems. **Population and Environment**, 24: 369–385. https://doi.org/10.1023/A:1023690824045

Matthews, D.M. (1983). Intestinal absorption of peptides. **Biochemical Society Transactions,** 11(6):808–10. https://doi.org/10.1152/physrev.1975.55.4.537

Meier, M.S., Stoessel, F., Jungbluth, N., Juraske, R., Schader, C., Stolze, M. (2015). Environmental impacts of organic and conventional agricultural products—Are the differences captured by life cycle assessment? **Journal of Environmental Management**, 149, 193–208. https://doi.org/10.1016/j.jenvman.2014.10.006

Minet R, Villie F, Marcollet M, Meynial-Denis D, Cynober L. (1997). Measurement of glutamine synthetase activity in rat muscle by a colorimetric assay. **Clinica Chimica Acta**, 268(1–2): 121–32. https://doi.org/10.1016/s0009-8981(97)00173-3

Miska, K.B., Fetterer, R.H. (2019). Expression of amino acid and sugar transporters, aminopeptidase, and the di- and tri-peptide transporter PepT1, differences between modern fast growing broilers and broilers not selected for rapid growth. **Poultry Science**, 98: 2272–2280. https://doi.org/10.3382/ps/pey583

Moreira, C.A, Bonagúrio, L.P, Esteves, L.A.C, Sitanaka, N.Y, Pozza, P.C. (2022). Dietary net energy mainly affects growth performance and pork quality of finishing pigs. **Scientia Agricola**, 79(1): e20190257. https://doi.org/10.1590/1678-992x-2019-0257

Moss AF, Liu SY, Selle PH. (2018a). Progress in comprehending the phytateephytase axis in chicken-meat production. **Animal Production Science**, 58(10): 1767-1778. https://doi.org/10.1071/AN17594.

Moss AF, Sydenham CJ, Khoddami A, Naranjo VD, Liu SY, Selle PH. (2018b). Dietary starch influences growth performance, nutrient utilisation and digestive dynamics of protein and amino acids in broiler chickens offered low-protein diets. **Animal Feed Science and Technology**, 237: 55e67. https://doi.org/10.1016/j.aninu.2022.01.003

Mottet, A., Tempio, G. (2017). Global poultry production: Current state and future outlook and challenges. **World's Poultry Science Journal**, 73: 245–25. https://doi.org/10.1017/S0043933917000071

Namroud NF, Shivazad M, Zaghari M. (2008). Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. **Poultry Science**, 87(11): 2250–8. https://doi.org/10.3382/ps.2007-00499

Noblet J, Dubois S, Lasnier J, Warpechowski M, Dimon P, Carré B, van Milgen J, Labussière E. (2015). Fasting heat production and metabolic BW in group-housed broilers. **Animal**, 9(7): 1138-44. https://doi.org/10.1017/S1751731115000403

Organisation for Economic Cooperation and Development (OECD). (2018). **Oilseeds and Oilseed Products in OECD-FAO Agricultural Outlook 2018–2027**. OECD, Paris. https://doi.org/10.1787/agr_outlook-2018-7-en

Ospina-Rojas IC, Murakami AE, Duarte CRA, Eyng C, Oliveira CAL, Janeiro V. (2014). Valine, isoleucine, arginine and glycine supplementation of low-protein diets for broiler chickens during the starter and grower phases. **British Poultry Science**, 55(6): 766–73. https://doi.org/10.1080/00071668.2014.970125

Ospina-Rojas IC, Murakami AE, Moreira I, Picoli KP, RJB R, Furlan AC. (2013). Dietary glycine+serine responses of male broilers given low-protein diets with different concentrations of threonine. **British Poultry Science**, 54 (4): 486–93. https://doi.org/10.1080/00071668.2013.794257

Parrini, S.; Aquilani, C.; Pugliese, C.; Bozzi, R.; Sirtori, F. (2023). Soybean replacement by alternative protein sources in pig nutrition and its effect on meat quality. **Animals**, 13: 494. https://doi.org/10.3390/ani13030494

Pesti, G.M. (2009). Impact of dietary amino acid and crude protein levels in broiler feeds on biological performance. **Journal of Applied Poultry Research**, 18: 477–486. https://doi.org/10.3382/japr.2008-00105

Reeds, P. J., Burrin, D. G., Stoll, B., & van Goudoever, J. B. (2000). Role of the gut in the amino acid economy of the host**. Nestle Nutrition workshop series. Clinical & performance programme**, 3:25–46. https://doi.org/10.1159/000061799Rostagno,

Reeds, P.J., Burrin, D.G., Stoll, B., van Goudoever, J.B. (1999). Consequences and regulation of gut metabolism. EAAP publication 96. Proc VIIIth Inter Symp Protein Metab Nutr. Wageningen, p. 127–53.

Salway JG. (2018). The Krebs uric acid cycle: A forgotten Krebs cycle. **Trends in Biochemical Sciences**, 43(11): 847–9. https://doi.org/10.1016/j.tibs.2018.04.012

Selle, P.H., de Paula Dorigam, J.C., Lemme, A., Chrystal, P.V., Liu, S.Y. (2020). Synthetic and crystalline amino acids: alternatives to soybean meal in chicken-meat production. **Animals**, 10(4):729. https://doi.org/10.3390/ani10040729

Selle, P.H., Liu, S.Y. (2019). The Relevance of Starch and Protein Digestive Dynamics in Poultry. **Journal of Applied Poultry Research**, 28(3): 531-545. https://doi.org/10.3382/japr/pfy026

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., Rosales, M., de Haan, C. (2006). Livestock's Long Shadow: **Environmental Issues and Options**, Food & Agriculture Org.: Rome, Italy. https://www.fao.org/3/a0701e/a0701e.pdf

Stern, R.A., Mozdziak, P.E. (2019). Differential ammonia metabolism and toxicity between avian and mammalian species, and effect of ammonia on skeletal muscle: A comparative review. **Journal of Animal Physiology and Animal Nutrition**, 103: 774–85. https://doi.org/10.1111/jpn.13080

Tudorache, M., Custura, I., Van, I., Popa, M.A. (2015). Study about chicken carcass protein content. **Scientific Papers-Series D-Animal Science**, 58: 342–34.

van Harn, J., Dijkslag, M.A., van Krimpen, M.M. (2019). Effect of low protein diets supplemented with free amino acids on growth performance, slaughter yield, litter quality, and footpad lesions of male broilers. **Poultry Science**, 98(10):4868–77. https://doi.org/10.3382/ps/pez229

van Milgen, J. (2021). The role of energy, serine, glycine, and 1-carbon units in the cost of nitrogen excretion in mammals and birds, **Animal**, 15(5): 100213. https://doi.org/10.1016/j.animal.2021.100213.

Wilke, M. S., Lovering, A. L., Strynadka, N. C. (2005). β-Lactam antibiotic resistance: A current structural perspective. **Current Opinion in Microbiology,** 8, 525–533. https://doi.org/10.1016/j.mib.2005.08.016

Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. **Amino Acids,** 37:1–17. https://doi.org/10.1007/s00726-009-0269-0

Wu, G. (2014). Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. **Journal of Animal Science and Biotechnology**, 5: 1–12. https://doi.org/10.1186/2049-1891-5-34

Wu, G., Bazer, F.W., Cross, H.R. (2014) Land-based production of animal protein: Impacts, efficiency, and sustainability. **Annals of the New York Academy of Sciences**, 1328: 18–28. https://doi.org/10.1111/nyas.12566

Zuidhof, M. (2019). A review of dietary metabolizable and net energy: Uncoupling heat production and retained energy. **Journal of Applied Poultry Research**, 28(2): 231-241. https://doi.org/10.3382/japr/pfx062

Zwarycz, B., Wong, E.A. (2013). Expression of the peptide transporters PepT1, PepT2, and PHT1 in the embryonic and post-hatch chick. **Poultry Science**, 92(5):1314–21. https://doi.org/10.3382/ps.2012-02826

CHAPTER 2 - Response of broiler chickens to reduced protein diets of different ingredient combinations

This chapter was prepared according to the guideline of Animal Feed Science and Technology.

Response of broiler chickens to reduced protein diets of different ingredient combinations

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Highlights

- Crude protein can be reduced up to 20 g/kg in corn-based broiler diets
- Prudence is advised when reducing the CP content of wheat-corn or sorghum-wheat diets
- Fat deposition in broilers increases with reduced protein levels
- Reducing dietary protein reduces nitrogen excretion
- Dietary composition may not have impact on nitrogen excretion in broiler chickens

Abstract

The nutrient composition of poultry diets can vary depending on ingredient availability, potentially impacting broiler performance, especially when fed as reduced crude protein (**CP**) diets. A total of 2,304 male, one-day-old Ross 308 broiler chicks were arranged in a 3 x 3 factorial. The birds were fed a standard starter diet from 0 to 7 d. On d 8, they were randomly allotted to nine dietary treatments of eight replicates with 32 birds each. The experimental diets were of three ingredient combinations (Corn; wheat-corn; sorghum-wheat) with three **CP** levels (Control/standard **CP**; Moderate **CP** reduction [control minus 20 g/kg **CP**]; Strong **CP** reduction [control minus 40 g/kg **CP**]) at the grower I (230, 210, and 190 g/kg **CP**), grower II (215, 195, and 175 g/kg **CP**), and finisher (205, 185, and 165 g/kg **CP**) phases. The birds' feed conversion ratio (**FCR**) was similar and better for those fed control and moderately reduced protein during the early growth stage (8 - 28 d) but worsened as **CP** levels reduced during the finisher phase. Regardless of the diet, protein and fat intake decreased as dietary **CP** levels decreased, while fat deposition and ratio of protein and fat deposition to intake increased. Birds excreted less nitrogen as the **CP** level decreased. Corn-based diets had the highest carcass weight and breast yield for all three **CP** levels, while wheat-corn and sorghum-wheat had similar values, but only at control and moderately reduced **CP** levels. **CP** digestibility was higher for corn-based and wheat-corn diets and lower for control and moderately reduced **CP** levels. Corn-based diets had the highest energy digestibility, but the digestible energy of corn-based and sorghum-wheat diets was similar. While the amino acid (**AA**) digestibility is similar and lower for control and moderately reduced **CP** levels, corn had the best (highest) AA digestibility compared to wheat-corn and sorghum-wheat. The study revealed that birds fed with wheat-corn or sorghum-wheat diets had lower but similar

BWG, and irrespective of the dietary composition, there is no difference in **BWG** up to 20 g/kg **CP** reduction. However, the broilers fed a corn-based diet recorded the lowest (best) **FCR**. Therefore, the reduction of 20 g/kg of **CP** in broiler chickens' diets allows the reduction of nitrogen excretion without negatively impacting carcass yield, breast yield, and protein deposition of the broiler chickens.

Keywords: Amino acid, fat deposition, nitrogen excretion, protein deposition, reduced crude protein, soybean meal.

1. Introduction

The increase in chicken meat consumption has necessitated the intensification of poultry production (Ferket et al., 2002; Mallin and Cahoon, 2003; OECD/FAO, 2021). Consequently, the manure produced in poultry farms increased substantially, leading to environmental problems in some regions of the world (de Vries and de Boer, 2010; Leip et al., 2015; Meier et al., 2015; Steinfeld et al., 2006). These increased demands have also added more strains to the existing ever-high soybean demand (Greenhalgh et al., 2020; OECD, 2018), which is the primary source of protein in broiler diets due to its high crude protein (**CP**) content of 440 – 480 g/kg (Rostagno et al., 2017) and relatively well-balanced AA profile when compared to other alternative plant protein (Beski et al., 2015). Given this, developing reduced **CP** diets for broiler chickens may help reduce soybean-dependence and increase sustainability in poultry production.

Reducing the **CP** of broiler diets and supplementing with non-bound (crystalline) AA has been suggested (Chrystal et al., 2020a; Greenhalgh et al., 2020; Liu and Selle, 2017; Selle et al., 2020; Woyengo et al., 2023) to be one of the solutions to the increased nitrogen (**N**) excretion from poultry, and the available studies have shown progress. The diets are often formulated by reducing the amount of soybean meal and increasing the amount of cereals with higher inclusions of AAs, to meet the AA requirements of the broiler chickens. Although most non-bound crystalline AAs are expensive, the historical price for the commonly supplemented ones (methionine, lysine, and threonine) indicates that crystalline AAs have become affordable with increased demand (Liu et al., 2021). Although reducing crude protein of broiler diets is not new (Selle et al., 2020); however, studies on reduced protein in broilers have become more intensified recently. Also, adoption has been slow among producers, but this seems to be a matter of time, for multiple reasons. The response of broilers due to dietary crude protein reduction might change depending on the feed ingredients used (Heger and Pack, 1996), highlighting the necessity to understand this phenomenon better.

Several authors (Chrystal et al., 2020a; Greenhalgh et al., 2020; Liu and Selle, 2017; Selle et al., 2020; Woyengo et al., 2023) have extensively reviewed the effects of reduced-**CP** diets on broilers. Studies have consistently shown that reducing dietary **CP** levels negatively affects broiler growth performance and increases fat deposition. Chrystal et al. (2021) found reduced growth performance in broiler chickens fed wheat-based diets with **CP** levels reduced from 200 to 160 g/kg, while Selle et al. (2021) reported lower body weight gain and feed intake in broilers fed reduced **CP** wheat-based diets compared to corn-based diets. Similarly, Bregendahl et al. (2002) found that reducing **CP** from 210 to 170 g/kg in corn-soybean diets reduced broiler growth rate and feed efficiency. Liu et al. (2014) reported that protein was more digestible in corn-based diets than wheat and sorghum; however, sorghum had better digestibility than wheat. Chrystal et al. (2021) reported increased protein digestibility as the dietary protein level decreased. Namroud et al. (2008) found increased fat deposition and abdominal fat pad as the level of **CP** in broilers' diets decreased, thereby supporting the assertion that the excess starch from reduced **CP** diets is metabolized to fat in broiler chickens.

Given the diversity of ingredient combinations utilized for poultry feed formulation in different parts of the world, there is pressing need for research on reduced **CP** that considers these differences. In this study, we formulated diets of varied ingredients so that the responses of broilers due to **CP** reduction could be evaluated in a broader scenario. We hypothesize that the response of broiler chickens to dietary **CP** intake will vary slightly according to the main ingredient combinations of the feed (corn, wheat-corn, or sorghum-wheat). Therefore, the present study aimed to evaluate the response (growth performance, carcass yield, nitrogen excretion, body composition and protein digestibility) of broiler chickens to different ingredient combinations while reducing the dietary **CP**.

2. Materials and methods

This study was conducted at the Poultry Science Laboratory of the School of Agricultural and Veterinary Sciences, São Paulo State University (UNESP). All procedures were approved by the Animal Care and Use Committee of UNESP (Protocol nº 639/22).

2.1 Experimental birds, housing, and management

A total of 2,304 one-day-old male Ross 308 broiler chicks weighing 42g ± 0.5 were obtained from a commercial hatchery. All birds were vaccinated against Marek and Pox virus on d 1 from the hatchery. On arrival, the birds were sexed and randomly distributed into pens containing wood shavings as litter, nipple drinkers, and tubular feeders. The poultry house was equipped with exhaust fans and pad cooling system which were used to automatically maintain the temperature, humidity, and air speed, as per the breeder guidelines (Aviagen, 2018).

On d 8, the birds were individually weighed and randomly distributed into nine dietary treatments of eight replicates each, making a total of 72 experimental units (pens) with 32 birds each, ensuring that the average weight of the birds in each pen did not differ from one another. Each pen has a dimension of 2.0 x 1.5 m, and a stocking density of approximately 11 birds/m^2 was maintained. Birds were given *ad libitum* access to water and feed throughout the experimental period. Lighting was provided to the birds continuously until seven days of age. On d 8, an 18h light : 6h dark lighting schedule was initiated, and this was maintained till the end of the experiment. On d 14 post-hatch, they were vaccinated against Infectious Bursal and Newcastle Diseases. Birds were not vaccinated against coccidiosis, but coccidiostat was included in their diets.

2.2 Experimental diets

The birds were fed a standard starter diet that meets the guideline recommendation of (Aviagen, 2019) from 0 to 7 d. On d 8 post-hatch, nine isocaloric (**AMEn**; MJ/kg) experimental diets were fed to the birds. The feeds were formulated to contain three **CP** levels (Control **CP**; Moderate **CP** reduction [control minus 20 g/kg **CP**]; Strong **CP** reduction [control minus 40 g/kg **CP**]) and three ingredient combinations (Corn; wheat and corn; sorghum and wheat). All other nutrients in the experimental feeds meet or exceed the Aviagen (2019) nutrient specification guide. A three-phase feeding strategy was employed, so that the treatments will have similar nutrient profiles at the grower I (8 – 18 d), grower II (18 – 28 d), and finisher (28 – 38 d) phases. The control, moderate, and strongly reduced **CP** diets were formulated with targeted levels of

230, 210, and 190 g/kg **CP** during the grower I phase; 215, 195, and 175 g/kg **CP** during the grower II phase; and 205, 185, and 165 g/kg **CP** during the finisher phase, respectively.

The experimental diets formulated for the starter, grower I, grower II, and finisher phases are presented in Tables 1, 2, 3, and 4, respectively. An indigestible marker (Titanium dioxide) was added to the finisher diets of the broiler chickens from 33 d to 38 d at 5 g/kg. Dry matter (**DM**), **CP**, **AMEn**, ether extract, and AA analyses of all organic feed ingredients were determined by Near-Infrared Spectroscopy (NIRS) by AMINONIR® (Evonik Operations GmbH, Germany) before formulating each diet, and the result of these analyses was employed in the diet formulations. After feed production at each phase, 200 g feed samples of each experimental diet were collected in a plastic bag, stored, and sent to AMINOLab® (Evonik Operations GmbH, Germany) to determine the **DM**, **CP**, gross energy (**GE**), and AAs. The AAs were determined using the High Performance Liquid Chromatography (HPLC), while **CP** was determined by Dumas' method. The analyzed AA content of the diets (total) at grower I, grower II, and finisher phases are presented in Table 5.

2.3 Growth performance

Bodyweight (**BW**), body weight gain (**BWG**), and feed intake (**FI**) were recorded on days 8, 18, 28, and 38. The feed conversion ratio (**FCR**) was calculated from the values of **BWG** and **FI**. The **FCR** was corrected daily for mortality.

2.4 Body composition

The body composition follows the comparative slaughter method of Sakomura & Rostagno (2016). On d 7 post-hatch, 12 reference birds were randomly selected to obtain the initial body composition before the commencement of the experiment, while on d 40 post-hatch, one bird per replicate, whose weight is close to the average weight of the group was selected. The birds were fasted for 24 hours, weighed, then euthanized via CO_2 asphyxiation.

After the collection of feather samples, the birds were scalded, plucked, then weighed to determine the plucked weight, after which they were stored at -20°C. The frozen bodies were cut (Delta Grill DG-1003, Brazil) and ground into pastes using a meat grinder (CAF 22 DSM, Brazil). The ground carcass was homogenized, sampled, and stored in an Ultra freezer (ColdLab CL374-86V, USA) for 24 hours, then lyophilized (Edwards Supermudulyo-220 freeze dryer, USA) at a temperature of -80°C.

The lyophilized carcass samples were ground using a ball grinder (Marconi MA 350, Brazil) and then analyzed for **DM**, ether extract, and crude protein. The feathers were dried to a constant weight in a convection oven for 72 hours to determine the **DM**, then ground (Tecnal - TE 631/3, Brazil) and analyzed for **CP**. The carcasses were first analyzed for ether extract (AOAC, 2005: method 920.39) to de-fat the samples (Ankom^{XT15} Extractor, USA) before being analyzed for protein. One g of the feather and carcass samples were weighed (Ohaus Adventurer AR2140, USA) and dried in a convection oven at 105°C for 16 h (AOAC, 2005: method 920.39) to determine the second **DM**. The **DM** from both readings were multiplied and divided by 100, to obtain the definite **DM**. Protein was determined by Kjeldahl procedure (AOAC, 2005: method 2001.11) and the gross energy was determined by total controlled combustion in an adiabatic bomb calorimeter (IKA C2000 basic, Germany).

Protein deposition (**Pd**) was calculated for both the body and feather then summed up, while the fat deposition (**Fd**) was calculated for the carcasses of both the reference and final birds as shown below. The final **Pd** and **Fd** of the chickens were calculated by subtracting the values measured on day 8 (reference) from that measured on day 41 (final), divided by the number of days. The protein and fat intake were also calculated, and the values were used to calculate the efficiencies of protein (**kP**) and fat (**kF**) depositions.

2.5 Nitrogen excretion

The **N** content of the diet was multiplied by the feed intake to obtain the nitrogen intake ($\mathbf{N}_{\boldsymbol{i}}$), while the **N** in the broiler carcass and feather was multiplied by the **BW** and feather weight, then added together to obtain the nitrogen retained ($\mathbf{N}_{\boldsymbol{R}}$) in the broiler chickens. These values were reported per day. The $\mathbf{N}_{\boldsymbol{R}}$ value was subtracted from the N_i to calculate the apparent nitrogen excretion ($\mathbf{AN}_{\boldsymbol{e}}$) of the broiler chickens according to the method of Bregendahl et al. (2002). The $\mathbf{AN}_{\boldsymbol{e}}$ relative to the $\mathbf{N}_{\boldsymbol{i}}$ i.e., $\mathbf{AN}_{\boldsymbol{e}}$ / $\mathbf{N}_{\boldsymbol{i}}$, was calculated to obtain the apparent nitrogen excretion coefficient ($\mathbf{AN}_{\boldsymbol{e}}\mathbf{C}$) of the broiler chickens.

2.6 Carcass traits

On day 39, three birds per replicate whose body weights were close to the average weight of the group were selected, fasted for 24 hours, weighed, then euthanized via cervical dislocation to evaluate carcass yields (total carcass yield, breast yield, and abdominal fat). The birds were then bled, scalded, plucked, and eviscerated. The head, neck, and feet were removed, after which the carcasses were weighed to obtain the carcass weight which was used to calculate the carcass yield. The breast (without skin) was removed and weighed for breast yield determination. The abdominal fat was also collected and weighed to determine the relative weight of the abdominal fat to live weight. The carcass yield, liver, and abdominal fats were recorded relative to the live weight, while breast weight (without skin) was calculated relative to the carcass weight (without shanks, head, and neck).

2.7 Apparent ileal nutrient digestibility coefficients

On day 40, a total of five birds per replicate were euthanized via cervical dislocation to collect the contents of the ileum using the flushing method by flushing the ileum with water. The digesta were collected from the final 2/3 distal part (cut about 5cm away from the ileocecal junction, towards the Meckel's diverticulum, and away from the Meckel's diverticulum, 5cm towards the caecum) of the intestine following the method of Sakomura and Rostagno (2016). The digesta was stored in an Ultra freezer (ColdLab CL374-86V, USA) for 24 hours at -85ºC, then lyophilized (Edwards Supermudulyo-220 freeze dryer, USA) at a temperature of -80ºC. The lyophilized digesta samples were ground using a ball grinder (Marconi MA 350, Brazil), and analyzed for **DM** (AOAC, 2005: method 920.39), **CP** (AOAC, 2005: method 2001.11), and GE (IKA C2000 basic, Germany), while the AAs in both digesta and diet were determined by AMINOLab® (Evonik Operations GmbH, Germany).

The indigestible marker (Titanium dioxide) in both the feed and digesta was recovered according to the method of Myers et al. (2004) with a slight adjustment to the digestion duration. The samples (0.25 g) were weighed (Ohaus Adventurer AR2140, USA) into 100 ml test tubes, with one empty tube serving as the blank. 3 g of $CuSO_4$ (catalyst) and 7 ml of H_2SO_4 were added, and the solutions were digested at 420ºC (temperature was gradually increased from 200ºC to 300ºC, and finally to 420ºC at a 30-minute interval, then left to digest for 2 hours or until the solution finally turns light green). The digested samples were allowed to cool for 30 minutes, after which 5 ml H_2O_2 was added into the test tubes. The solution was poured into a beaker and distilled water was added to take the volume to 50 ml, and then filtered using Whatman® 541 filter papers. The filtered solutions were read using a UV/VIS spectrophotometer (Beckman DU 640; USA) at 410 nm. The absorbances were corrected for the equation ($Y = 0.1702x - 0.0183$; $R^2 = 0.9997$) obtained from the curve. The digestibility was calculated using a formula adapted from Sakomura and Rostagno (2016).

$$AIDC = \frac{(N_{diet}/\ TiO_{2\ diet}) - \ (N_{dig.}/TiO_{2\ dig.})}{(N_{diet}/\ TiO_{2\ diet})}$$

Where **AIDC** is Apparent ileal digestibility coefficient (Energy, **CP**, and AAs [methionine, cysteine, lysine, threonine, tryptophan, arginine, isoleucine, leucine, valine, histidine, phenylalanine, glycine, serine, proline, alanine, aspartate, and glutamate]), $\mathbf{N}_{diet}$ is nutrient or energy in diet, $\mathbf{N}_{dig.}$ is nutrient or energy in digesta, $\mathbf{TiO_2}_{\ diet}$ is the titanium dioxide concentration in the diet, $\mathbf{TiO_2}_{\ dig.}$ is the titanium dioxide concentration in the digesta.

Digestible energy (**DE**) was calculated according to Gautier & Rochell (2020),

$$DE = GE_{diet} * ED$$

Where $\mathbf{GE}_{diet}$ is the gross energy in diet while **ED** is the energy digestibility coefficient.

2.8 Statistical analysis

All the data obtained from this study were checked for normality and homoscedasticity of errors using the Cramer-Von Mises and Brown and Forsythe tests at 5%, respectively. Outliers were removed only after checking for plausibility. The data were analyzed as a one-way ANOVA in a 3 x 3 experimental arrangement using the SAS® ODA Software (SAS Institute Inc., Cary, NC, USA). Means were compared using Tukey test at 5% level of probability. At the grower II and finisher phases, **BW** at d 18 and **BW** at d 28 were used as covariates, respectively, for analyses of the growth performance parameters.

3. Results

3.1 Growth performance

The growth performance of broiler chickens fed reduced **CP** diets of varying ingredient sources at different growth phases is presented in Table 6.

3.1.1 Grower I phase (8 to 18 d)

During the grower I phase (8 – 18d), the interaction between diet and CP levels did not have significant ($P > 0.05$) effect on **BW** and **BWG** of the broiler chickens. However, **FI** ($P = 0.007$) and **FCR** ($P = 0.028$) were significantly affected by the interaction between diets and **CP** level. The **FI** of birds fed with corn or sorghum-wheat diets was consistent across all **CP** levels. In contrast, birds fed with wheat-corn diet at control **CP** level had 5.7% more **FI** than those fed with strongly reduced **CP** diet. The **FCR** of broiler chickens fed with corn or sorghum-wheat diets was lower for control and moderately reduced **CP** levels compared with those fed strongly reduced **CP** diets, while the birds fed with wheat-corn diet showed no differences between the **CP** levels evaluated.

3.1.2 Grower II phase (18 to 28 d)

In this phase, the interaction between diets and **CP** levels did not have significant ($P >$ 0.05) effect on **BW**, **BWG**, and **FCR**, but significantly ($P = 0.047$) affected the **FI**. The **FI** of broiler chickens fed with corn or sorghum-wheat diets were similar across the evaluated **CP** levels. Nonetheless, the birds fed with wheat-corn diet at strongly reduced **CP** level exhibited higher (5.3%) **FI** compared with those fed control **CP**.

The **BW**, **BWG**, and **FCR** were significantly influenced ($P < 0.05$) by diets and **CP** levels. Broiler chickens fed with corn-based diets showed higher **BWG** ($P = 0.009$) compared with those fed sorghum-wheat diets. Interestingly, the birds fed wheat-corn diets had similar **BWG** to both corn and sorghum-wheat diets. However, corn yielded 3.8% lower **FCR** ($P < 0.001$) than those fed wheat-corn or sorghum-wheat diets.

3.1.3 Finisher phase (28 to 38 d)

During the finisher phase, the **BW**, **BWG**, and **FI** of the broiler chickens were significantly ($p<0.05$) influenced by the interaction of diet and **CP** levels. While the **BW**, **BWG** and **FI** seemed to be similar across all treatments, the **BWG** of the birds fed wheat-corn or sorghum-wheat diets were 8.8% and 12.2%, respectively, lower than those fed corn-based diets.

The **FCR** of the birds fed with corn-based diet was 5.5% and 4.3% lower ($P < 0.001$) than those fed wheat-corn or sorghum-wheat diets, respectively, while that of birds fed control **CP** was higher than those fed with moderately or strongly reduced **CP** levels. The similarity ($P < 0.05$) in **FCR** values for control and moderately reduced **CP** levels at the grower I and grower II phases, while different ($P < 0.05$) at the finisher phase could indicate that 20 g/kg **CP** reduction poses no threat to growth performance of broiler chickens at earlier stages of growth but could be debilitating at latter stages. It could also be a result of the ingredient combinations employed in this study.

3.1.4 Cumulative growth performance (8 to 38 d)

The cumulative growth performance result (Table 7) shows that all the parameters evaluated (**BW**, **BWG**, **FI**, and **FCR**) were not significantly (*P* > 0.05) influenced by the interaction between diets and **CP** levels. However, the diets and CP levels had significant (*P* < 0.05) effect on **BW**, **BWG**, **FI**, and **FCR**.

The **BW** and **BWG** of birds fed with corn-based diet were higher compared with those fed with wheat-corn or sorghum-wheat diets. Broiler chickens fed with corn-based diets showed **FI** similar to those fed with sorghum-wheat diet, but 2% lower than those fed with wheat-corn diet on average. Furthermore, the **FCR** of birds fed with corn-based diet was lowest, followed by those fed with sorghum-wheat, while the birds fed with wheat-corn diets showed the highest **FCR**.

Although reducing the **CP** level by 20 g/kg (medium) did not have a negative effect on the **BW**, **BWG**, and **FI** of broiler chickens, as their values were comparable to those fed the control **CP**, the **FCR** varied significantly (*P* < 0.05) among the three **CP** levels. Specifically, the **FCR** increased as the **CP** level decreased.

3.2 Body composition

Table 8 shows the body composition of broiler chickens fed reduced **CP** diets of varying ingredient sources. It was observed that the interaction of diets and **CP** levels had significant (*P* < 0.05) impact on all the evaluated parameters, except protein deposition. Protein and fat intake reduced (*P* < 0.05) as the levels of **CP** reduce for all diet types, although birds fed with wheat-corn diets at control and moderately reduced **CP** levels have similar fat intake. Also, birds fed with sorghum-wheat diets at strongly reduced **CP** level had the lowest protein intake.

The fat deposition of broiler chickens was observed to increase as the level of **CP** decreased, for all diet types. The fat deposition increased by 14.2%, 7.9%, and 16.8%, from control to moderately reduced **CP**, in corn, wheat-corn, and sorghum-wheat diets, respectively. Wheat-corn diets therefore seem to deposit less fat compared with corn or sorghum-wheat.

For all diet types, ratio of protein deposition to intake of the broiler chickens increased ($P < 0.05$) with decreased **CP** level. The broiler chickens fed with corn-based diets at control **CP** level had lower protein deposition efficiency ($P = 0.011$) than those of moderately or strongly reduced **CP** levels. The birds fed with wheat-corn diets had similar ratio of protein deposition to intake across all **CP** levels. Similarly, for all diet types, the ratio of fat deposition to intake also increased ($P < 0.05$) as the dietary **CP** level reduced. Broiler chickens fed corn or sorghum-wheat diets at strongly reduced **CP** level showed lower fat deposition efficiency than those fed moderately or strongly reduced **CP** levels. However, the fat deposition efficiency of broiler chickens fed wheat-corn diet at control and medium **CP** levels were similar and higher for those fed strongly reduced **CP** levels.

The CP levels significantly ($P < 0.05$) influenced the protein deposition in the broiler chickens. The protein deposition reduces ($P < 0.05$) as the **CP** levels decreases, although the values recorded for control (13.16 g/b*d) and moderately reduced (12.91 g/b*d) **CP** levels are statistically ($P < 0.05$) similar, which follows the result of the growth performance where the control had similar ($P < 0.05$) result to that of moderately reduced **CP** level.

3.3 Nitrogen excretion

The interaction between diets and **CP** levels had no significant ($P > 0.05$) effect on the evaluated parameters (Table 9), except nitrogen intake ($P < 0.001$). Irrespective of the diet type, the nitrogen intake decreased progressively as the **CP** level decreased. Although, the nitrogen intake of birds fed sorghum-wheat diet at strongly reduced **CP** level recorded was lowest, even compared to the low **CP** level of other diets.

The **CP** level significantly ($P < 0.05$) impacted the apparent nitrogen excretion, nitrogen retention, and the apparent nitrogen excretion coefficient. The apparent nitrogen excretion decreased ($P < 0.0001$) as the **CP** level decreased, as the broiler chickens fed with control **CP** level excreted the highest amount of nitrogen and lowest with those of strongly reduced **CP** level. The nitrogen retained was similar for birds fed control or moderately reduced **CP** but higher than that of strongly reduced **CP** level. Interestingly, the coefficient of apparent nitrogen excretion was lower and similar for birds fed moderately or strongly reduced **CP** compared to control **CP** diets, indicating that birds tend to excrete more **N** with increased **N** intake.

3.4 Carcass traits

The carcass yield and liver were not significantly ($P > 0.05$) affected by the interaction between diets and **CP** levels (Table 10). However, the fasted body weight, carcass weight, abdominal fat, and breast yield of broiler chickens were influenced ($P < 0.05$) by the interaction between diets and **CP** level. Birds fed corn or wheat-corn diets at control or moderately reduced **CP** levels had higher ($P < 0.05$) fasted body weight than those fed strongly reduced **CP** diets. In contrast, broiler chickens fed sorghum-wheat diet at control **CP** level showed higher fasted body weight than birds fed strongly or moderately reduced **CP** levels.

The carcass weight of broiler chickens fed all diet types were similar for control or moderately reduced **CP**, while the lowest ($P < 0.05$) value was observed for those fed strongly reduced **CP** levels. The abdominal fat of broiler chickens increased ($P < 0.001$) as the **CP** level decreased, with corn or sorghum-wheat diets at control and moderately reduced **CP** levels having lower abdominal fat than those fed strongly reduced **CP** diets. The breast yield was similar across all treatment interactions, with the lowest yield observed in birds fed sorghum-wheat of strongly reduced **CP** levels. Birds fed corn-based diets had similar breast yield for all **CP** levels despite reduction of **CP** up to 40 g/kg (strongly reduced **CP**), while wheat-corn and sorghum-wheat only recorded similar values for control and moderately reduced **CP** levels (up to 20 g/kg) only. The relative liver weight (P = 0.003) of birds fed corn-based diet was lower than those fed wheat-corn diet, while the value recorded for the sorghum-wheat diet was similar to the other two diets. Control and moderately reduced dietary **CP** resulted in lower relative liver weight compared with birds fed strongly reduced **CP** levels.

3.5 Apparent ileal nutrient digestibility coefficients

3.5.1 CP and energy

The **CP** digestibility coefficient, energy digestibility coefficient, and digestible energy of the broiler chickens were not significantly ($P > 0.05$) influenced by the interaction between diets and **CP** levels (Table 11). However, diet type influenced ($P < 0.05$) all parameters evaluated, while the **CP** levels influenced ($P < 0.001$) **CP** digestibility. The **CP** digestibility of broiler chickens fed corn or wheat-corn diets was similar and higher compared with birds fed with sorghum-wheat diet. Also, birds fed control and moderately reduced **CP** levels showed lower **CP** digestibility compared with those whose **CP** levels were strongly reduced. The energy digestibility coefficient of broiler chickens fed corn-based diet was lower compared with those fed wheat-corn or sorghum-wheat diets. In contrast, broilers fed corn-based, or sorghum-wheat diets had higher and similar digestible energy compared with the birds fed wheat-corn diet.

3.5.2 Amino acids

While the apparent digestibility of eight (methionine, lysine, arginine, leucine, histidine, glycine, serine, and aspartate) of the 16 AAs considered in this study were not significantly (P > 0.05) influenced by the interaction between diets and **CP** levels. Eight (cysteine, threonine, isoleucine, valine, phenylalanine, proline, alanine, and glutamate) were significantly (P < 0.05) impacted (Tables 12.1 and 12.2). Across all diets, the digestibility coefficients of cysteine (P = 0.0441) and alanine (P = 0.0499) were similar for birds fed at control, moderate, or strong **CP** reduction. For all the AA digestibility coefficients of broiler chickens affected by the interaction of diet and **CP** levels, corn had similar and best digestibility at all **CP** levels. Birds fed wheat-corn diets at control and moderate **CP** reduction yielded similar results for the aforementioned AAs. However, threonine, isoleucine, valine, and glutamate digestibilities were significantly different, and increased as the **CP** level reduced for the birds fed sorghum-wheat diet.

Corn had the best (highest) digestibility coefficient for seven of the AAs that were not significantly influenced by the interaction of diet and **CP** level. While the digestibility coefficients of birds fed wheat-corn and sorghum-wheat were similar but lower for methionine, lysine, arginine, and aspartate, the digestibilities of leucine, histidine, and serine were different (P < 0.05) for the three diet types, with birds fed sorghum-wheat having the poorest digestibility coefficient for leucine, histidine, and serine.

The birds fed control and moderately reduced **CP** levels had similar (P < 0.05) but lower methionine, lysine, arginine, and glycine digestibilities, compared with those fed low **CP** diets. Although control and moderate **CP** reduction have different digestibilities compared to strongly reduced **CP** diets, the difference was not huge, and only amounted to 2%, 3.2%, 1%, and 4.5%, for methionine, lysine, arginine, and glycine, respectively.

4. Discussion

Corn has been reported to be the best cereal when feeding reduced **CP** diets to broiler chickens (Chrystal et al., 2021; Liu et al., 2021). The current study corroborates those reports. Although at the early stages (8 – 28 d post-hatch), the **FI** and **FCR** seemed similar for birds fed all diet types investigated herein, up to 20 g/kg CP reduction. It could be argued that the presence of kafirin and condensed tannin in sorghum (Liu et al., 2015) and the non-starch polysaccharide (NSP) fractions in wheat (Ravindran and Amerah, 2009) are part of the reasons for the discrepancies in responses, but the similarity in the early-stage performance leave more questions unanswered. This leaves the discrepancy in protein digestibility as the most probable reason, as Liu et al. (2014) reported that protein (nitrogen) in corn-based diets is 5% and 9% more digestible than in wheat-based and sorghum-based diets, respectively, in the distal ileum. It could therefore be explained that the available protein for utilization for both the wheat-corn and sorghum-wheat diets was able to sustain the birds' growth at an earlier age, but could no longer keep up when they grew older, hence the seemingly poorer performance at the finisher phase, when compared to that of corn.

Liu et al. (2021) reported that **CP** can be reduced in broiler chicken diets up to 3% and this was supported by the study of Chrystal et al. (2021) when they found that birds fed 2.9% reduced dietary **CP** had similar **FI** and **FCR** to those fed control **CP**. Although our result corroborates the studies when the broiler chickens were young (d 8 – 28), the slightly increased feed intake of the wheat-corn medium **CP** diet at the finisher phase caused a slight discrepancy in the feed conversion ratio, thus resulting in statistically different **FCR** across all **CP** levels at the finisher (28 – 38 d) phase and overall (8 – 38 d) period. Although our result for growth performance does not totally resonate with the reports of Chrystal et al. (2021) and Liu et al. (2021), it is similar to the result of some studies (Maynard et al., 2021; van Harn et al., 2019) that found differences in the **FCR** of birds fed diets of up to 20 g/kg and 40 g/kg **CP** reduction. Chrystal et al. (2021) designed their experiment to have only wheat or corn at every point in time, not a combination, which was also the view of Liu et al. (2021), while the other experiments (Maynard et al., 2021; van Harn et al., 2019) had ingredient combinations (wheat and corn) similar to our study, although van Harn et al. (2019) supplemented their diets with enzymes, while Maynard et al. (2021) found their differences from 8 – 28d post-hatch. Therefore, the results could be attributed to the ingredient combinations employed in this study. Considering the result obtained for the cumulative growth performance where the **BW**, **BWG**, and **FI** were similar for control and moderate dietary **CP** reduction, and other parameters (carcass yield, nitrogen excretion, digestibility, protein deposition, and the ratio of protein deposition to intake) of this study supporting 20 g/kg **CP** reduction, the result of the **FCR** alone might not be enough to conclude that 20 g/kg **CP** reduction is not sustainable.

The reduction of protein and fat intake as dietary **CP** reduces is expected, as the **FI** seems to remain almost constant with reducing dietary **CP** levels (Table 6), at the same time, fat composition of the diet also reduced (Tables 2, 3, and 4). Thus, it is reasonable that a reduction in dietary protein and fat content will lead to a reduction in their intake. The reduction in the protein deposition as the **CP** level decreased follows the trend of the growth performance at early stages (d 8 – 28 post-hatch), where the values recorded for control (13.16 g/b*d) and moderately reduced (12.91 g/b*d) **CP** levels were statistically similar. Reducing the dietary **CP** by 40 g/kg yielded the lowest protein deposition in the birds. Waldroup (2007) argued that the poor performance of broilers fed reduced **CP** diets could be attributed to an insufficient nitrogen pool for the synthesis of non-essential amino acids or amino acid imbalances and deamination of excess amino acids, which results in the accumulation of excessive ammonia (Selle et al., 2020). Although all essential amino acid requirements were met in the current study, the lower protein deposition recorded with the birds fed the lowest **CP** level could be a result of the imbalances that could ensue.

Li et al. (2019) opined that extra glucose produced after the consumption of an excessive amount of carbohydrates by mammals is used to create glycogen, which is then stored in the liver. However, if the glucose concentration is more than necessary to produce glycogen and energy, the leftover glucose molecules are converted to fat (Zhang et al., 2010). Thus, excess starch (glucose) borne from the reduction of dietary **CP** of broiler chickens is converted to fat. This process with other fates of dietary glucose under conditions of chronically high energy is well described by Alemany (2011) and Li et al. (2019). According to Gous et al. (2007), broiler chickens fed reduced **CP** diets consume more energy than protein and deposit the excess energy as lipid. The progression of the fat deposition with reducing **CP** level follows the trend of the abdominal fat in the study, which is reasonable, as the reduction in dietary **CP** reduces dietary fat (and decreased heat increment [**HI**]) but increases dietary starch composition as shown in the diet composition for the broiler chickens (Tables 2, 3, and 4). The result of the present study is similar to those presented by several authors (Allameh and Toghyani, 2019; Chrystal et al., 2021, 2020b; Hilliar et al., 2020) that reported increased abdominal fat pad, and also to that of Namroud et al. (2008) who found increased fat deposition and abdominal fat pad with decreased dietary **CP** in broiler chickens' diets. For all diet types (ingredient combinations), the increased ratio of protein deposition to intake with decreased **CP** level could be attributed to the need for the broiler chickens to improve the efficiency of utilizing the dietary protein as the protein in the diet diminishes. The ratio of fat deposition to intake also increased as the dietary **CP** level reduces, and most of the values are above 1, which results from lower fat intake compared to deposition (**Fd**), thus buttressing the fact that excess starch from reduced **CP** diets is metabolized to fat in broiler chickens.

Protein deposition in broiler chickens is limited by their genetic potential, and overconsumed **N** is excreted (Woyengo et al., 2023). Also, Selle et al. (2020) suggested that excessive AA intake by broilers, as a result of high **CP** or otherwise, results in deamination of the excess AAs, thus leading to increased excretion of **N** as uric acid in manure. In this study, the **N** excretion was not determined from the manure, but rather calculated as an apparent excretion from the retention and intake. However, the apparent **N** excretion of the broiler chickens reduced as the level of dietary **CP** reduces. Reducing dietary **CP** of broiler chickens by 20 g/kg and 40 g/kg in this study reduced the apparent nitrogen excretion by 19% and 31%, respectively. This result corroborates several studies (Hofmann et al., 2019; Lemme et al., 2019; Macelline et al., 2020; van Harn et al., 2019), showing that reducing dietary **CP** in broiler nutrition is one of the ways to reduce the contribution of poultry to global warming, as opined by Liu et al. (2021). Although the apparent **N** excretion reduces with reduced **CP** level, the coefficient of apparent **N** excretion (relative to **N** intake) in this study only differs between control and reduced **CP** diets. There was no significant difference between the **N** excretion of the two reduced **CP** levels. This implies that lesser **N** is excreted from 1 unit of **N** intake as dietary protein reduces, which supports the overall aim of reduced protein in broiler diets.

The impact of reduced dietary **CP** on the carcass yield of broiler chickens has been reported in several studies (Brink et al., 2022; Hilliar et al., 2020; Lemme et al., 2019; van Harn et al., 2019), albeit with consideration of different yield parameters. In this study, for all three ingredient combinations (diet types), it was found that the live and carcass weights are similar for control and moderately reduced **CP** levels. This follows the trend recorded for the **BWG** of the growth performance, thus supporting the fact that 20 g/kg protein reduction, if supplemented with the limiting AAs, is not inimical. Although, the carcass yield was not significantly affected by diet and **CP** level interaction, the highest yields were recorded for control and moderate **CP** reduction, compared to strongly reduced **CP** level. The breast yielded similar values for corn-based diets despite the reduction of dietary **CP** up to 40 g/kg. In comparison, wheat-corn and sorghum-wheat only recorded similar values for control and medium **CP** levels (up to 20 g/kg reduction). This once again supports the claim of Chrystal et al. (2021) that a corn-based diet is best for reduced **CP** in broiler chickens.

Not many reports are available on the liver of broiler chickens fed reduced **CP**. In our study, the liver increased as the **CP** level decreased, which could infer an increased workload borne from the metabolism of excess starch (glucose) to fatty acids that occur in the liver due to higher inclusion of grains in reduced **CP** diets. Regardless of the diet type, the abdominal fat of the broiler chickens increased as the **CP** level decreased, indicating that the increased starch resulting from decreased **CP** resulted in increased fat deposition. The steady increase in abdominal fat with decreasing **CP** level goes together with the fat deposition result (discussed above) in this study and is similar to the studies of Allameh and Toghyani (2019), Chrystal et al. (2021, 2020b), Hilliar et al. (2020), Namroud et al. (2008), among others. Despite formulating the diet with similar apparent metabolizable energy values, we observed that the effective energy (**EE**) values of the diets differ (increased as the **CP** level decreases), due to the decrease in the heat increment of the diets. The **EE** is similar to the net energy (**NE**) system (Barzegar et al., 2020). It has been shown in pigs (Batorek-Lukač et al., 2021; Moreira et al., 2021), and laying hens (Barzegar et al., 2019), that fat deposition increases with increased **NE**. Perhaps, formulating reduced **CP** diets in broilers using either the **EE** or **NE** system (considering the optimal value of the control **CP** group as standard) may be helpful to combat the increased fat deposition of reduced **CP** diets in broiler nutrition, thus allowing more energy for transamination and deamination of excess AAs (Namroud et al., 2008), rather than starch metabolism to fat.

Corn-based and wheat-corn-based diets had better protein digestibility coefficients than sorghum-wheat diets in broiler chickens. Liu et al. (2014) found that protein was more digestible in corn compared to wheat and sorghum, while sorghum had better digestibility than wheat. The better digestibility recorded for corn by the authors is similar to our findings, but the better result obtained for the wheat-based diet compared to sorghum could be a result of the small amount of corn present in the wheat-corn diet, or perhaps the residual effect of the **CP** reduction across the treatments. Although the apparent **CP** digestibility increases as the **CP** level reduces, the control and moderately reduced **CP** levels still have statistically similar values. Chrystal et al. (2021) found that reducing the protein level of broiler chicken diets by 57 g/kg caused a significant improvement in the protein digestibility of the diets. The result of the present study suggests that reducing dietary protein up to 20 g/kg in broiler diets may not be enough to elicit improvement in protein digestibility, since Chrystal et al. (2021) started finding significant change at 29 g/kg **CP** reduction level, while that of this study commenced at 40 g/kg **CP** reduction. While the apparent energy digestibility of corn is better than the other feed ingredients tested in this study, wheat-corn and sorghum-wheat had similar apparent digestibility values for energy. However, the digestible energy values for corn and sorghum-wheat diets later proved similar to wheat-corn.

The similar AA (cysteine, threonine, isoleucine, valine, phenylalanine, proline, alanine, and glutamate) digestibility coefficients recorded for birds fed corn-based diets at either control, moderate, or strong **CP** reduction, compared with the other diets supports the superiority of corn while feeding reduced **CP** diets to broiler chickens. It has been demonstrated that the ileal digestibilities of isoleucine, valine, and phenylalanine were best for corn, compared with sorghum or wheat Lemme et al. (2004). However, wheat had the best digestibility for cysteine and threonine. The slight dip in the digestibilities of cysteine, threonine, isoleucine, valine, phenylalanine, proline, alanine, and glutamate for wheat-corn at moderately reduced **CP** level could be the reason for the slightly increased **FI** recorded for the birds of the same group at the finisher phase, as they perhaps wanted to consume more diet to meet up their needs, thereby inadvertently increasing the **FCR**.

The increased AA digestibilities observed with decreased **CP** levels observed in this study has reported by several studies using either corn-based (Chrystal et al., 2020b, 2020c), wheat-corn (Chrystal et al., 2021), or wheat-sorghum (Hilliar et al., 2020) diets. Chrystal et al. (2020c) attributed this to the increased unbound proportion in the formulation as dietary **CP** reduces, compared to the protein-bound proportion. Hilliar et al. (2020) reported that this reflects the increased inclusion of feed-grade crystalline AAs in reduced **CP** diets. While the arguments are relevant, there is also a possibility that the need for the birds to meet up with requirement could have pushed them to improve nutrient utilization, and consequently **CP** digestibility in the reduced **CP** diets. Although, control and moderate **CP** reduction in the current study yielded similar results, which corresponds with the growth performance, carcass trait, nitrogen excretion, **CP** digestibility, and protein deposition results, indicating that the birds could not have struggled with 20 g/kg **CP** reduction.

The results of the study indicate that corn remains the best-suited energy source for reduced **CP** broiler diet. While the fat deposition of broiler chickens fed a sorghum-wheat diet with a 20 g/kg **CP** reduction was comparable to those fed corn or wheat-corn diets of the same **CP** level, the cumulative **FCR** of the sorghum-wheat diet was slightly better than that of the wheat-corn diet. Despite this, the carcass weight and breast yield of both diets (wheat-corn and sorghum-wheat) were similar with a 20 g/kg **CP** reduction. Therefore, although their yields are not comparable to corn, it is possible to feed wheat-corn or sorghum-wheat diets to broiler chickens at a 20 g/kg **CP** reduction. Reduction of 20 g/kg in dietary **CP** reduced soybean meal inclusion, on average, by 20.57%, 27.24%, and 23.23%, for corn, wheat-corn, and sorghum-wheat diets, respectively. Although profit is the main driving factor of the poultry industry, **FCR** may not be the only factor to consider when concluding on the optimal level to which **CP** can be reduced in broiler diets.

5. Conclusion

The reduction of 20 g/kg in dietary crude protein allows the reduction of nitrogen excretion without negatively impacting carcass yield, breast yield, crude protein and amino acid digestibilities, and protein deposition of the broiler chickens. Birds fed with wheat-corn or sorghum-wheat diets had lower but similar body weight gain, while the broilers fed corn-based diet recorded the lowest (best) feed conversion ratio.

CRediT authorship contribution statement

Damilola U. Kareem: Investigation, Methodology, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Juliano Cesar P. Dorigam**: Conceptualization, Project administration, Methodology, Funding acquisition, Writing - review & editing. **Matheus P. Reis**: Conceptualization, Methodology, Writing - review & editing. **Lucas P. Bonagurio**: Data curation, Formal analysis, Writing - review & editing. **Audasley T. Fialho**: Investigation, Formal Analysis. **Larissa M. Pereira**: Investigation, Formal Analysis. **Rosiane S. Camargos**: Investigation, Formal Analysis. **Bruno B. Leme**: Investigation, Formal Analysis. **Nilva K. Sakomura**: Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Writing - review & editing.

Declaration of interests

None

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References

Alemany, M., 2011. Utilization of dietary glucose in the metabolic syndrome. Nutr Metab (Lond) 8, 1–10. https://doi.org/10.1186/1743-7075-8-74

Allameh, S., Toghyani, M., 2019. Effect of dietary valine supplementation to low protein diets on performance, intestinal morphology and immune responses in broiler chickens. Livest Sci 229, 137–144. https://doi.org/10.1016/J.LIVSCI.2019.09.025

AOAC, 2005. Official Method of Analysis, 18th ed. Association of Official Analytical Chemistry, Arlington, USA.

Aviagen, 2019. Ross Broiler - Nutrition Specifications. Aviagen, Huntsville, Alabama, USA.

Aviagen, 2018. Ross Broiler Pocket Guide: The Pocket Guide. Aviagen, Huntsville, Alabama, USA.

Barzegar, S., Wu, S.B., Choct, M., Swick, R.A., 2020. Factors affecting energy metabolism and evaluating net energy of poultry feed. Poult Sci 99, 487–498. https://doi.org/10.3382/ps/pez554

Barzegar, S., Wu, S.B., Noblet, J., Choct, M., Swick, R.A., 2019. Energy efficiency and net energy prediction of feed in laying hens. Poult Sci 98, 5746–5758. https://doi.org/10.3382/PS/PEZ362

Batorek-Lukač, N., Čandek-Potokar, M., Škrlep, M., Kubale, V., Labussière, E., 2021. Effect of Changes in Dietary Net Energy Concentration on Growth Performance, Fat Deposition, Skatole Production, and Intestinal Morphology in Immunocastrated Male Pigs. Front Vet Sci 8, 1481. https://doi.org/10.3389/FVETS.2021.789776/BIBTEX

Beski, S.S.M., Swick, R.A., Iji, P.A., 2015. Specialized protein products in broiler chicken nutrition: A review. Animal Nutrition 1, 47–53. https://doi.org/10.1016/J.ANINU.2015.05.005

Bregendahl, K., Sell, J.L., Zimmerman, D.R., 2002. Effect of Low-Protein Diets on Growth Performance and Body Composition of Broiler Chicks. Poult Sci 81, 1156–1167. https://doi.org/https://doi.org/10.1093/ps/81.8.1156

Brink, M., Janssens, G.P.J., Demeyer, P., Bağci, Ö., Delezie, E., 2022. Reduction of dietary crude protein and feed form: Impact on broiler litter quality, ammonia concentrations, excreta composition, performance, welfare, and meat quality. Animal Nutrition 9, 291–303. https://doi.org/10.1016/j.aninu.2021.12.009

Chrystal, P. V, Greenhalgh, S., Mcinerney, B. V, Mcquade, L.R., Akter, Y., Cesar, J., Dorigam, P., Selle, P.H., Liu, S.Y., 2021. Maize-based diets are more conducive to crude protein reductions than wheat-based diets for broiler chickens. Anim Feed Sci Technol 275, 114867. https://doi.org/10.1016/j.anifeedsci.2021.114867

Chrystal, P. V., Greenhalgh, S., Selle, P.H., Liu, S.Y., 2020a. Facilitating the acceptance of tangibly reduced-crude protein diets for chicken-meat production. Animal Nutrition 6, 247–257. https://doi.org/10.1016/j.aninu.2020.06.001

Chrystal, P. V., Moss, A.F., Khoddami, A., Naranjo, V.D., Selle, P.H., Liu, S.Y., 2020b. Effects of reduced crude protein levels, dietary electrolyte balance, and energy density on the performance of broiler chickens offered maize-based diets with evaluations of starch, protein, and amino acid metabolism. Poult Sci 99, 1421–1431. https://doi.org/10.1016/J.PSJ.2019.10.060

Chrystal, P. V., Moss, A.F., Khoddami, A., Naranjo, V.D., Selle, P.H., Liu, S.Y., 2020c. Impacts of reduced-crude protein diets on key parameters in male broiler chickens offered maize-based diets. Poult Sci 99, 505–516. https://doi.org/10.3382/ps/pez573

de Vries, M., de Boer, I.J.M., 2010. Comparing environmental impacts for livestock products: A review of life cycle assessments. Livest Sci 128, 1–11. https://doi.org/10.1016/j.livsci.2009.11.007

Ferket, P.R., van Heugten, E., van Kempen, T.A.T.G., Angel, R., 2002. Nutritional strategies to reduce environmental emissions from nonruminants. J Anim Sci 80, E168–E182. https://doi.org/10.2527/animalsci2002.80e-suppl_2e168x

Gautier, A.E., Rochell, S.J., 2020. Influence of coccidiosis vaccination on nutrient utilization of corn, soybean meal, and distillers dried grains with solubles in broilers. Poult Sci 99, 3540–3549. https://doi.org/10.1016/j.psj.2020.03.035

Gous, R.M., Emmans, G.C., Broadbent, L.A., Fisher, C., 1990. Nutritional effects on the growth and fatness of broilers. Br Poult Sci 31, 495–505. https://doi.org/10.1080/00071669008417281

Greenhalgh, S., Chrystal, P. V., Selle, P.H., Liu, S.Y., 2020. Reduced-crude protein diets in chicken-meat production: justification for an imperative. Worlds Poult Sci J 76, 537–548. https://doi.org/10.1080/00439339.2020.1789024

Heger, J., Pack, M. (Biofaktory P. (Czech R., 1996. Effects of dietary glycine + serine on starting broiler chick performance as influenced by dietary crude protein levels. Agribiological research (Germany) 49, 257–265.

Hilliar, M., Hargreave, G., Girish, C.K., Barekatain, R., Wu, S.B., Swick, R.A., 2020. Using crystalline amino acids to supplement broiler chicken requirements in reduced protein diets. Poult Sci 99, 1551–1563. https://doi.org/10.1016/J.PSJ.2019.12.005

Hofmann, P., Siegert, W., Kenéz, Á., Naranjo, V.D., Rodehutscord, M., 2019. Very low crude protein and varying glycine concentrations in the diet affect growth performance, characteristics of nitrogen excretion, and the blood metabolome of broiler chickens. J Nutr 149, 1122–1132. https://doi.org/10.1093/JN/NXZ022

Leip, A., Billen, G., Garnier, J., Grizzetti, B., Lassaletta, L., Reis, S., Simpson, D., Sutton, M.A., De Vries, W., Weiss, F., Westhoek, H., 2015. Impacts of European livestock production: Nitrogen, sulphur, phosphorus and greenhouse gas emissions, land-use, water eutrophication and biodiversity. Environmental Research Letters 10. https://doi.org/10.1088/1748-9326/10/11/115004

Lemme, A., Hiller, P., Klahsen, M., Taube, V., Stegemann, J., Simon, I., 2019. Reduction of dietary protein in broiler diets not only reduces n-emissions but is also accompanied by several further benefits. J Appl Poult Res 28, 867–880. https://doi.org/10.3382/JAPR/PFZ045

Lemme, A., Ravindran, V., Bryden, W.L., 2004. Ileal digestibility of amino acids in feed ingredients for broilers. Worlds Poult Sci J 60, 423–438. https://doi.org/10.1079/WPS200426

Li, Z., Li, J., Liu, X.L., Liu, D.D., Li, H., Li, Z.J., Han, R.L., Wang, Y.B., Liu, X.J., Kang, X.T., Yan, F.B., Tian, Y.D., 2019. Effects of different starch sources on glucose and fat metabolism in broiler chickens Effects of different starch sources on glucose and fat metabolism in broiler chickens. Br Poult Sci 60, 449–456. https://doi.org/10.1080/00071668.2019.1605150

Liu, S.Y., Cadogan, D.J., Péron, A., Truong, H.H., Selle, P.H., 2014. Effects of phytase supplementation on growth performance, nutrient utilization and digestive dynamics of starch and protein in broiler chickens offered maize-, sorghum- and wheat-based diets. Anim Feed Sci Technol 197, 164–175. https://doi.org/10.1016/J.ANIFEEDSCI.2014.08.005

Liu, S.Y., Fox, G., Khoddami, A., Neilson, K.A., Truong, H.H., Moss, A.F., Selle, P.H., 2015. Grain Sorghum: A Conundrum for Chicken-Meat Production. Agriculture (Switzerland) 5, 1224–1251. https://doi.org/10.3390/agriculture5041224

Liu, S.Y., Macelline, S.P., Chrystal, P. V., Selle, P.H., 2021. Progress towards reduced-crude protein diets for broiler chickens and sustainable chicken-meat production. J Anim Sci Biotechnol 12, 1–13. https://doi.org/10.1186/s40104-021-00550-w

Liu, S.Y., Selle, P.H., 2017. Starch and protein digestive dynamics in low-protein diets supplemented with crystalline amino acids. Anim Prod Sci 57, 2250–2256. https://doi.org/10.1071/AN17296

Macelline, S.P., Wickramasuriya, S.S., Cho, H.M., Kim, E., Shin, T.K., Hong, J.S., Kim, J.C., Pluske, J.R., Choi, H.J., Hong, Y.G., Heo, J.M., 2020. Broilers fed a low protein diet supplemented with synthetic amino acids maintained growth performance and retained intestinal integrity while reducing nitrogen excretion when raised under poor sanitary conditions. Poult Sci 99, 949–958. https://doi.org/10.1016/J.PSJ.2019.10.035

Mallin, M.A., Cahoon, L.B., 2003. Industrialized animal production - A major source of nutrient and microbial pollution to aquatic ecosystems. Popul Environ 24, 369–385. https://doi.org/10.1023/A:1023690824045

Maynard, C.W., Ghane, A., Chrystal, P. V., Selle, P.H., Liu, S.Y., 2021. Sustaining live performance in broilers offered reduced crude protein diets based on corn and wheat blend. Anim Feed Sci Technol 276. https://doi.org/10.1016/j.anifeedsci.2021.114928

Meier, M.S., Stoessel, F., Jungbluth, N., Juraske, R., Schader, C., Stolze, M., 2015. Environmental impacts of organic and conventional agricultural products - Are the differences captured by life cycle assessment? J Environ Manage 149, 193–208. https://doi.org/10.1016/j.jenvman.2014.10.006

Moreira, C. de A., Bonagúrio, L.P., Esteves, L.A.C., Sitanaka, N.Y., Pozza, P.C., 2021. Dietary net energy mainly affects growth performance and pork quality of finishing pigs. Sci Agric 79, e20190257. https://doi.org/10.1590/1678-992X-2019-0257

Myers, W.D., Ludden, P.A., Nayigihugu, V., Hess, B.W., 2004. Technical Note: A procedure for the preparation and quantitative analysis of samples for titanium dioxide. J Anim Sci 82, 179–183. https://doi.org/10.2527/2004.821179x

Namroud, N.F., Shivazad, M., Zaghari, M., 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult Sci 87, 2250–2258. https://doi.org/10.3382/PS.2007-00499

OECD, 2018. Oilseeds and oilseed products, in: OECD-FAO Agricultural Outlook 2018-2027. pp. 127–138. https://doi.org/10.1787/agr_outlook-2018-7-en

OECD/FAO, 2021. Meat, in: OECD-FAO AGRICULTURAL OUTLOOK 2021-2030. pp. 163–177.

Ravindran, V., Amerah, A.M., 2009. Wheat: composition and feeding value for poultry, in: Davies, S., Evans, G. (Eds.), Soybean and Wheat Crops. Nova Science Publishers, Inc., pp. 245–258.

Rostagno, H.S., Albino, L.F.T., Hannas, M.I., Donzele, J.L., Sakomura, N.K., Perazzo, F.G., Saraiva, A., de Abreu, M.L.T., Rodrigues, P.B., de Oliveira, R.F., Barreto, S.L. de T., Brito, C.O., 2017. Brazilian Tables for Poultry and Swine: Feedstuff Composition and Nutritional Requirements, 4th ed.

Sakomura, N.K., Rostagno, H.S., 2016. Métodos de pesquisa em nutrição de monogástricos, 2nd ed. Fundação de Apoio a Pesquisa, Ensino e Extensão (FUNEP), Jaboticabal.

Selle, P.H., Cantor, D.I., McQuade, L.R., McInerney, B. V., de Paula Dorigam, J.C., Macelline, S.P., Chrystal, P. V., Liu, S.Y., 2021. Implications of excreta uric acid concentrations in broilers offered reduced crude protein diets and dietary glycine requirements for uric acid synthesis. Animal Nutrition 7, 939–946. https://doi.org/10.1016/j.aninu.2021.03.011

Selle, P.H., De Paula Dorigam, J.C., Lemme, A., Chrystal, P. V., Liu, S.Y., 2020. Synthetic and crystalline amino acids: Alternatives to soybean meal in chicken-meat production. Animals 10, 1–20. https://doi.org/10.3390/ani10040729

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M., Rosales, M.M., de Haan, C., 2006. Livestock's Long Shadow: Environmental Issues and Options, Food and Ariculture Organisation. Food and Ariculture Organisation, Rome, Italy.

van Harn, J., Dijkslag, M.A., Van Krimpen, M.M., 2019. Effect of low protein diets supplemented with free amino acids on growth performance, slaughter yield, litter quality, and footpad lesions of male broilers. Poult Sci 98, 4868–4877. https://doi.org/10.3382/ps/pez229

Waldroup, P.W., 2007. Do crude protein levels really matter?, in: 15th Annual ASAIM Southeast Asian Feed Technology and Nutrition Workshop. Indonesia, pp. 1–5.

Woyengo, T.A., Knudsen, K.E.B., Børsting, C.F., 2023. Low-protein diets for broilers: Current knowledge and potential strategies to improve performance and health, and to reduce environmental impact. Anim Feed Sci Technol 297, 115574. https://doi.org/10.1016/j.anifeedsci.2023.115574

Zhang, L.Z., Zhao, R.J., Liu, J.K., Gao, S.T., Zhu, T.N., 2010. Effects of Pyrrolidine Dithiocarbamate on Synthesis of Hepatic Glycogen in Diabetic Rats. Chinese Journal of Pathophysiology 26, 2442–2446.

Tables

Table 1. Composition of experimental diet at the starter phase (0 to 8 d)

Ingredients	g/kg
Corn, grain	325.70
Sorghum grain, low tannin	100.00
Wheat, grain	100.00
Soybean oil	38.10
Peanut meal	23.30
Soybean meal	370.80
Dicalcium Phosphate	18.30
Limestone	8.10
Salt (NaCl)	2.80
PX.Minerals[1]	1.00
PX.Vitamins[1]	1.00
DL-Methionine (990)[2]	3.50
L-Lysine HCl (780)	2.10
L-Threonine (985)	0.90
Choline chloride, 600 g/kg	1.00
Coccidiostat	0.50
Potassium carbonate	0.60
Sodium bicarbonate	1.30
Adsorbent	1.00
Total	1000.00
Calculated nutritional composition, g/kg unless noted otherwise	
Crude Protein	243
Digestible protein	216
AMEn (MJ/kg)	12.4
NE (MJ/kg)[3]	11.0
Crude Fiber	34.1
Ether Extract	68.2
Starch	333.2
Ash	40.6
Calcium	9.0
Available Phosphorus	4.5
Sodium (Na)	1.6
Chlorine (Cl)	2.3
Potassium (K)	9.2
SID Lysine	12.6
SID Methionine (Met)	6.4
SID Met.+Cyst	9.3
SID Threonine	8.1
SID Tryptophan	2.6
SID Arginine	14.8
SID Valine	9.7
SID Isoleucine	9.0
SID Leucine	16.9

SID Histidine	5.4
SID Phenylalanine (Phe)	10.6
SID Phe+Tyr	18.4
SID Cysteine (Cys)	3.0
SID Tyrosine (Tyr)	7.7
SID Glycine (Gly)	8.2
SID Serine (Ser)	10.5
SID Gly+Ser	18.8
Electrolyte Balance (mEq/kg)	239.8
Analyzed amino acid composition (Total), g/kg	
Methionine	6.7
Cysteine	3.7
Met+Cys	10.3
Lysine	14.3
Threonine	9.2
Tryptophan	ND
Arginine	16.3
Isoleucine	10.6
Leucine	19.7
Valine	11.3
Histidine	6.1
Phenylalanine	12.1
Glycine	10.0
Serine	11.2
Proline	14.3
Alanine	11.1
Aspartate	24.1
Glutamate	44.0

[1] Provided per kg of vitamin premix: Folic Acid (min) 1600 mg; Vitamin B5 - Pantothenic acid (min) 24.96 g; Biotin (min) 80 mg; Butyl hydroxide toluene 100 mg; Niacin (min) 67.20 g; Selenium (min) 600 mg; Vitamin A (min) 13440000 UI; Vitamin B1 (min) 3492 mg; Vitamin B12 (min) 19200 mcg; Vitamin B2 (min) 9600 mg; Vitamin B6 (min) 4992 mg; Vitamin D3 (min) 3200000 UI; Vitamin K3 (min) 2880 mg. Provided per kg of mineral premix: Copper (min) 15 g; Iron (min) 90 g; Iodine (min) 1500 mg; Manganese (min) 150 g; Zinc (min) 140 g.

[2] Purities (g/kg) of non-bound amino acids are included in parentheses.

[3] **EE (kcal/kg) = AMEn - (*wd* * FOM) – (0.16 * *wu* * DCP) + (12 * *z* * DCL)** [Emmans et al., 1994]

Where, EE = Effective energy; AMEn = Apparent metabolizable energy corrected to zero nitrogen retention; ***wd*** = 3.80; FOM = Feed organic matter; ***wu*** = 29.2; DCP = Digestible crude protein; ***z*** = 0.3; DCL = Digestible crude lipid

SID - Standard ileal digestibility; **ND** – Not determined

Table 2. Composition of experimental diet at the grower I phase (8 to 18 d)

Ingredients (g/kg)	Corn (g/kg)			Wheat and corn (g/kg)			Sorghum and wheat (g/kg)		
	Control CP	Moderate CP reduction	Strong CP reduction	Control CP	Moderate CP reduction	Strong CP reduction	Control CP	Moderate CP reduction	Strong CP reduction
Corn, grain	531.40	596.80	669.70	285.00	285.00	285.00	-	-	-
Sorghum grain, low tannin	-	-	-	-	-	-	377.70	450.60	546.20
Wheat, grain	-	-	-	287.00	368.30	456.40	200.00	200.00	200.00
Soybean oil	39.40	27.40	13.40	46.70	35.90	22.00	47.10	35.10	17.20
Peanut meal	70.00	70.00	70.00	-	-	-	-	-	-
Meat and bone meal	-	-	-	-	-	-	20.00	20.00	20.00
Soybean meal	313.70	253.50	183.80	336.20	257.10	165.70	317.10	246.70	148.10
Dicalcium Phosphate	17.00	17.40	17.80	16.80	17.10	17.60	11.30	11.80	12.40
Limestone	7.80	8.00	8.30	7.70	8.10	8.50	5.50	5.70	6.10
Salt (NaCl)	2.70	2.70	2.60	2.80	2.70	2.70	2.70	2.70	2.70
Premix (Minerals)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix (Vitamins)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine (990)[2]	3.00	3.50	4.00	2.70	3.20	3.70	3.00	3.60	4.40
L-Lysine HCl (780)	1.90	3.70	5.80	1.80	4.00	6.60	2.20	4.40	7.30
L-Threonine (985)	0.70	1.40	2.30	0.60	1.60	2.70	0.90	1.80	3.10
L-Valine (965)	-	1.00	2.10	-	1.10	2.40	0.10	1.20	2.80
L-Isoleucine (904)	-	0.60	1.70	-	0.50	1.80	-	0.60	2.20
Glycine (985)	-	-	1.70	-	-	2.60	-	0.30	3.70
L-Arginine (900)	-	-	0.40	-	0.70	3.10	-	1.20	4.10
L-Tryptophan (980)	-	-	0.20	-	-	0.20	-	-	0.20
L-Phenylalanine (966)	-	-	-	-	-	1.80	-	-	1.90
L-Histidine (900)	-	-	-	-	-	0.10	-	-	0.70
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coccidiostat	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Inert	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium carbonate	1.60	3.10	5.00	1.80	3.80	6.20	2.00	3.80	6.40
Sodium bicarbonate	1.30	1.40	1.50	1.40	1.50	1.60	0.80	0.80	0.90
Adsorbent	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutritional composition, g/kg unless noted otherwise									
Crude Protein	230	210	190	230	210	194	230	210	190

Digestible protein	206	187	169	199	178	161	195	177	159
AMEn (MJ/kg)	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6	12.6
EE (MJ/kg)[2]	11.3	11.4	11.5	11.3	11.5	11.6	11.4	11.5	11.7
Crude Fiber	33.0	30.9	28.5	32.4	29.6	26.4	30.9	28.4	24.7
Ether Extract	72.2	61.4	48.6	74.2	62.9	48.3	75.3	63.8	46.3
Starch	347.5	389.4	436.1	344.4	389.4	438.2	358.6	405.9	468.0
Ash	38.7	35.8	32.3	37.6	33.9	29.6	40.7	37.4	32.6
Calcium	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4	8.4
Available Phosphorus	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2	4.2
Sodium (Na)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Chlorine (Cl)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Potassium (K)	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
SID Lysine	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5	11.5
SID Methionine (Met)	5.7	5.9	6.2	5.5	5.7	5.9	5.8	6.0	6.4
SID Met.+Cyst	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
SID Threonine	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4	7.4
SID Tryptophan	2.3	2.0	1.9	2.5	2.1	1.9	2.5	2.1	1.9
SID Arginine	15.1	13.4	11.9	13.2	11.9	11.9	12.7	11.9	11.9
SID Valine	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1	9.1
SID Isoleucine	8.3	7.9	7.9	8.5	7.9	7.9	8.4	7.9	7.9
SID Leucine	16.2	14.9	13.4	15.4	13.6	11.6	15.9	14.5	12.4
SID Histidine	5.3	4.7	4.2	5.1	4.5	3.9	4.8	4.1	3.9
SID Phenylalanine (Phe)	10.2	9.2	7.9	9.9	8.7	9.0	9.8	8.5	8.7
SID Phe+Tyr	17.8	15.9	13.8	16.7	14.4	13.4	16.7	14.6	13.4
SID Cysteine (Cys)	2.8	2.6	2.3	3.0	2.8	2.6	2.7	2.5	2.1
SID Tyrosine (Tyr)	7.6	6.8	5.9	6.8	5.6	4.4	6.9	6.0	4.7
SID Glycine (Gly)	8.1	7.3	8.0	7.7	6.8	8.2	8.0	7.3	9.0
SID Serine (Ser)	10.0	8.9	7.7	10.0	8.9	7.5	9.6	8.4	6.7
SID Gly+Ser	18.1	16.2	15.7	17.8	15.7	15.7	17.6	15.7	15.7
Electrolyte Balance (mEq/kg)	240	240	240	240	240	240	240	240	240

[1] Provided per kg of vitamin premix: Folic Acid (min) 1600 mg; Vitamin B5 - Pantothenic acid (min) 24.96 g; Biotin (min) 80 mg; Butyl hydroxide toluene 100 mg; Niacin (min) 67.20 g; Selenium (min) 600 mg; Vitamin A (min) 13440000 UI; Vitamin B1 (min) 3492 mg; Vitamin B12 (min) 19200 mcg; Vitamin B2 (min) 9600 mg; Vitamin B6 (min) 4992 mg; Vitamin D3 (min) 3200000 UI; Vitamin K3 (min) 2880 mg. Provided per kg of mineral premix: Copper (min) 15 g; Iron (min) 90 g; Iodine (min) 1500 mg; Manganese (min) 150 g; Zinc (min) 140 g.

[2] Purities (g/kg) of non-bound amino acids are included in parentheses.

[3] **EE (kcal/kg) = AMEn - (*wd* * FOM) – (0.16 * *wu* * DCP) + (12 * *z* * DCL)** [Emmans et al., 1994]

Where, EE = Effective energy; AMEn = Apparent metabolizable energy corrected to zero nitrogen retention; ***wd*** = 3.80; FOM = Feed organic matter; ***wu*** = 29.2; DCP = Digestible crude protein; ***z*** = 0.3; DCL = Digestible crude lipid

SID - Standard ileal digestibility

Table 3. Composition of experimental diet at the grower II phase (18 to 28 d)

Ingredients (g/kg)	Corn (g/kg)			Wheat and corn (g/kg)			Sorghum and wheat (g/kg)		
	Control CP	Moderate CP reduction	Strong CP reduction	Control CP	Moderate CP reduction	Strong CP reduction	Control CP	Moderate CP reduction	Strong CP reduction
Corn, grain	564.00	625.70	693.90	250.00	250.00	250.00	-	-	-
Sorghum grain, low tannin	-	-	-	-	-	-	410.20	476.60	567.60
Wheat, grain	-	-	-	364.80	444.40	537.90	200.00	200.00	200.00
Soybean oil	45.40	34.40	21.40	54.80	44.50	29.80	53.90	43.40	26.80
Peanut meal	70.00	70.00	70.00	-	-	-	-	-	-
Meat and bone meal	-	-	-	-	-	-	20.00	20.00	20.00
Soybean meal	278.00	221.60	157.80	287.50	210.40	114.40	280.70	217.50	124.80
Dicalcium Phosphate	15.10	15.40	15.90	14.80	15.20	15.60	9.40	9.80	10.40
Limestone	7.20	7.40	7.70	7.20	7.60	8.00	4.90	5.10	5.50
Salt (NaCl)	2.70	2.70	2.60	2.80	2.70	2.70	2.70	2.70	2.70
Premix (Minerals)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix (Vitamins)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine (990)[2]	2.40	2.80	3.30	2.10	2.50	3.10	2.40	2.90	3.70
L-Lysine HCl (780)	1.50	3.10	5.00	1.60	3.70	6.50	1.80	3.70	6.50
L-Threonine (985)	0.30	1.10	1.90	0.40	1.30	2.50	0.60	1.40	2.60
L-Valine (965)	-	0.50	1.50	-	0.70	2.10	-	0.70	2.20
L-Isoleucine (904)	-	0.30	1.30	-	0.30	1.70	-	0.30	1.80
Glycine (985)	-	-	0.70	-	-	2.00	-	-	2.60
L-Arginine (900)	-	-	-	-	0.60	3.10	-	0.80	3.50
L-Tryptophan (980)	-	-	0.20	-	-	0.10	-	-	0.10
L-Phenylalanine (966)	-	-	-	-	-	1.90	-	-	1.20
L-Histidine (900)	-	-	-	-	-	0.10	-	-	0.50
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coccidiostat	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Inert	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium carbonate	2.50	4.00	5.70	3.00	5.00	7.50	3.00	4.60	7.10
Sodium bicarbonate	1.40	1.50	1.60	1.40	1.50	1.60	0.80	0.80	0.90
Adsorbent	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutritional composition, g/kg unless noted otherwise									
Crude Protein	215	195	175	215	195	177	215	195	175

Digestible protein	191	174	155	182	161	143	181	163	145
AMEn (kcal/kg)	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0
EE (kcal/kg)[2]	11.7	11.8	11.9	11.8	11.9	12.0	11.8	11.9	12.1
Crude Fiber	31.6	29.7	27.5	30.6	27.9	24.5	29.5	27.2	23.8
Ether Extract	78.6	68.7	57.0	81.1	70.3	54.9	82.1	72.1	55.9
Starch	368.4	408.0	451.6	365.1	409.2	461.0	379.7	422.8	481.9
Ash	36.2	33.4	30.3	34.6	31.0	26.5	38.2	35.2	30.8
Calcium	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Available Phosphorus	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Sodium (Na)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Chlorine (Cl)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Potassium (K)	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
SID Lysine	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3
SID Methionine (Met)	5.0	5.2	5.4	4.7	4.9	5.1	5.0	5.3	5.6
SID Met.+Cyst	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
SID Threonine	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.6
SID Tryptophan	2.1	1.9	1.7	2.3	2.0	1.7	2.3	2.0	1.7
SID Arginine	14.1	12.5	10.8	12.0	10.7	10.7	11.7	10.7	10.7
SID Valine	8.5	8.2	8.2	8.5	8.2	8.2	8.4	8.1	8.1
SID Isoleucine	7.7	7.1	7.1	7.9	7.1	7.1	7.8	7.1	7.1
SID Leucine	15.4	14.2	12.8	14.3	12.6	10.4	15.1	13.8	11.9
SID Histidine	4.9	4.5	3.9	4.8	4.2	3.5	4.4	3.8	3.5
SID Phenylalanine (Phe)	9.6	8.6	7.4	9.2	8.1	8.4	9.1	8.0	7.6
SID Phe+Tyr	16.7	14.9	12.9	15.3	13.0	12.0	15.5	13.7	12.0
SID Cysteine (Cys)	2.7	2.5	2.2	2.9	2.7	2.5	2.6	2.3	2.0
SID Tyrosine (Tyr)	7.1	6.3	5.5	6.0	5.0	3.6	6.4	5.6	4.4
SID Glycine (Gly)	7.6	6.8	6.6	7.2	6.3	7.1	7.4	6.5	7.6
SID Serine (Ser)	9.3	8.4	7.3	9.4	8.2	6.8	9.0	7.9	6.3
SID Gly+Ser	17.0	15.2	13.9	16.6	14.6	13.9	16.4	14.4	13.9
Electrolyte Balance (mEq/kg)	240	240	240	240	240	240	240	240	240

[1] Provided per kg of vitamin premix: Folic Acid (min) 1600 mg; Vitamin B5 - Pantothenic acid (min) 24.96 g; Biotin (min) 80 mg; Butyl hydroxide toluene 100 mg; Niacin (min) 67.20 g; Selenium (min) 600 mg; Vitamin A (min) 13440000 UI; Vitamin B1 (min) 3492 mg; Vitamin B12 (min) 19200 mcg; Vitamin B2 (min) 9600 mg; Vitamin B6 (min) 4992 mg; Vitamin D3 (min) 3200000 UI; Vitamin K3 (min) 2880 mg. Provided per kg of mineral premix: Copper (min) 15 g; Iron (min) 90 g; Iodine (min) 1500 mg; Manganese (min) 150 g; Zinc (min) 140 g.

[2] Purities (g/kg) of non-bound amino acids are included in parentheses.

[3] **EE (kcal/kg) = AMEn - (*wd* * FOM) – (0.16 * *wu* * DCP) + (12 * *z* * DCL)** [Emmans et al., 1994]

Where, EE = Effective energy; AMEn = Apparent metabolizable energy corrected to zero nitrogen retention; ***wd*** = 3.80; FOM = Feed organic matter; ***wu*** = 29.2; DCP = Digestible crude protein; ***z*** = 0.3; DCL = Digestible crude lipid

SID - Standard ileal digestibility

Table 4. Composition of experimental diet at the finisher phase (28 to 38 d)

Ingredients (g/kg)	Corn (g/kg)			Wheat and corn (g/kg)			Sorghum and wheat (g/kg)		
	Control CP	Moderate CP reduction	Strong CP reduction	Control CP	Moderate CP reduction	Strong CP reduction	Control CP	Moderate CP reduction	Strong CP reduction
Corn, grain	582.20	644.00	712.00	230.00	230.00	230.00	-	-	-
Sorghum grain, low tannin	-	-	-	-	-	-	428.40	496.20	588.00
Wheat, grain	-	-	-	409.50	491.80	581.30	200.00	200.00	200.00
Soybean oil	50.30	39.30	26.30	60.90	50.10	35.60	59.30	48.40	31.50
Peanut meal	70.00	70.00	70.00	-	-	-	-	-	-
Meat and bone meal	-	-	-	-	-	-	20.00	20.00	20.00
Soybean meal	254.00	197.50	134.00	255.50	175.30	83.30	256.20	191.50	97.90
Dicalcium Phosphate	15.20	15.60	16.10	15.00	15.30	15.80	9.60	10.00	10.60
Limestone	7.30	7.50	7.80	7.40	7.70	8.10	5.00	5.20	5.60
Salt (NaCl)	2.70	2.70	2.60	2.70	2.70	2.70	2.70	2.70	2.70
Premix (Minerals)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Premix (Vitamins)[1]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
DL-Methionine (990)[2]	2.20	2.70	3.10	1.90	2.30	2.90	2.30	2.80	3.50
L-Lysine HCl (780)	1.60	3.20	5.10	1.80	4.10	6.70	1.90	3.90	6.70
L-Threonine (985)	0.30	1.10	1.90	0.50	1.40	2.50	0.60	1.40	2.60
L-Valine (965)	-	0.50	1.50	-	0.80	2.10	-	0.80	2.30
L-Isoleucine (904)	-	0.30	1.40	-	0.40	1.80	-	0.40	1.90
Glycine (985)	-	-	0.50	-	-	1.80	-	-	2.50
L-Arginine (900)	-	-	0.10	-	1.00	3.40	-	1.00	3.70
L-Tryptophan (980)	-	-	0.20	-	-	0.20	-	-	0.20
L-Phenylalanine (966)	-	-	-	-	-	2.30	-	-	1.50
L-Histidine (900)	-	-	-	-	-	0.20	-	-	0.60
Choline chloride, 600 g/kg	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Coccidiostat	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Inert[3]	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Potassium carbonate	3.20	4.70	6.40	3.90	5.90	8.30	3.70	5.30	7.80
Sodium bicarbonate	1.40	1.50	1.60	1.50	1.60	1.70	0.80	0.80	0.90
Adsorbent									
Total	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated nutritional composition, g/kg unless stated otherwise									

Crude Protein	205	185	165	205	185	168	205	185	165
Digestible protein	182	164	146	171	151	133	172	154	136
AMEn (kcal/kg)	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2	13.2
EE (kcal/kg)[4]	12.0	12.1	12.2	12.1	12.2	12.3	12.1	12.2	12.4
Crude Fiber	30.7	28.7	26.6	29.3	26.5	23.3	28.5	26.1	22.6
Ether Extract	83.6	73.7	62.0	86.3	75.0	59.9	87.4	77.0	60.5
Starch	380.0	419.6	463.2	377.0	422.6	472.1	391.5	435.5	495.1
Ash	34.9	32.2	29.0	33.1	29.3	25.0	37.0	33.9	29.4
Calcium	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Available Phosphorus	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8	3.8
Sodium (Na)	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Chlorine (Cl)	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3
Potassium (K)	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2	9.2
SID Lysine	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8	9.8
SID Methionine (Met)	4.7	4.9	5.1	4.4	4.6	4.8	4.8	5.0	5.4
SID Met.+Cyst	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2
SID Threonine	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
SID Tryptophan	2.0	1.7	1.6	2.2	1.8	1.6	2.2	1.9	1.6
SID Arginine	13.4	11.8	10.1	11.2	10.1	10.1	11.0	10.1	10.1
SID Valine	8.1	7.8	7.8	8.1	7.8	7.8	8.0	7.7	7.8
SID Isoleucine	7.3	6.7	6.7	7.4	6.7	6.7	7.4	6.7	6.7
SID Leucine	14.8	13.6	12.2	13.5	11.7	9.6	14.5	13.2	11.2
SID Histidine	4.7	4.2	3.7	4.5	3.9	3.3	4.2	3.6	3.3
SID Phenylalanine (Phe)	9.1	8.0	7.0	8.7	7.5	8.3	8.7	7.5	7.4
SID Phe+Tyr	15.9	14.1	12.2	14.3	12.0	11.4	14.7	12.8	11.4
SID Cysteine (Cys)	2.6	2.4	2.1	2.8	2.7	2.4	2.5	2.2	1.9
SID Tyrosine (Tyr)	6.7	6.0	5.2	5.6	4.4	3.1	6.1	5.3	4.1
SID Glycine (Gly)	7.3	6.5	6.1	6.8	5.9	6.5	7.1	6.1	7.1
SID Serine (Ser)	8.9	7.9	6.8	8.9	7.7	6.4	8.5	7.4	5.8
SID Gly+Ser	16.2	14.4	12.9	15.8	13.7	12.9	15.6	13.5	12.9
Electrolyte Balance (mEq/kg)	240	240	240	240	240	240	240	240	240

[1] Provided per kg of vitamin premix: Folic Acid (min) 1600 mg; Vitamin B5 - Pantothenic acid (min) 24.96 g; Biotin (min) 80 mg; Butyl hydroxide toluene 100 mg; Niacin (min) 67.20 g; Selenium (min) 600 mg; Vitamin A (min) 13440000 UI; Vitamin B1 (min) 3492 mg; Vitamin B12 (min) 19200 mcg; Vitamin B2 (min) 9600 mg; Vitamin B6 (min) 4992 mg; Vitamin D3 (min) 3200000 UI; Vitamin K3 (min) 2880 mg. Provided per kg of mineral premix: Copper (min) 15 g; Iron (min) 90 g; Iodine (min) 1500 mg; Manganese (min) 150 g; Zinc (min) 140 g.
[2] Purities (g/kg) of non-bound amino acids are included in parentheses.
[3] inert was added to the diet at the inclusion level of 5 g/kg from d 29 to 32 and replaced with titanium dioxide in the diet from d 33 to 38.
[4] **EE (kcal/kg) = AMEn - (*wd* * FOM) – (0.16 * *wu* * DCP) + (12 * *z* * DCL)** [Emmans et al., 1994]
Where, EE = Effective energy; AMEn = Apparent metabolizable energy corrected to zero nitrogen retention; ***wd*** = 3.80; FOM = Feed organic matter; ***wu*** = 29.2; DCP = Digestible crude protein; ***z*** = 0.3; DCL = Digestible crude lipid
SID - Standard ileal digestibility

Table 5. Analyzed amino acid composition (total) of the diets at grower I, grower II, and finisher phases

Amino acids	Corn			Wheat and Corn			Sorghum and wheat		
	Control CP[1]	Moderate CP reduction[2]	Strong CP reduction[3]	Control CP	Moderate CP reduction	Strong CP reduction	Control CP	Moderate CP reduction	Strong CP reduction
				Analyzed amino acid composition (Total), g/kg					
Grower I (8 – 18d)									
Methionine	6.3	6.5	6.5	6.1	6.0	6.8	6.0	7.0	6.8
Cysteine	3.4	3.2	3.0	3.8	3.5	3.2	3.4	3.1	2.7
Met+Cys	9.7	9.7	9.5	9.9	9.5	10.1	9.4	10.1	9.4
Lysine	13.3	13.3	12.3	13.3	12.8	13.3	12.9	13.1	12.6
Threonine	8.8	8.5	8.0	8.9	8.5	8.6	8.7	8.5	8.2
Tryptophan	2.7	2.4	2.2	2.9	2.5	2.2	2.8	2.4	2.1
Arginine	17.0	15.4	13.4	15.0	13.2	13.1	14.3	13.1	12.9
Isoleucine	9.7	9.2	8.8	10.1	17.8	15.0	9.7	9.0	8.8
Leucine	18.8	17.4	15.7	18.1	8.1	7.9	18.7	16.7	14.7
Valine	10.7	10.8	10.3	10.9	10.3	10.6	10.8	10.8	10.5
Histidine	6.0	5.5	4.8	5.9	5.1	4.4	5.4	4.7	4.3
Phenylalanine	11.6	10.4	9.0	11.5	10.0	10.6	11.3	9.8	10.0
Glycine	10.2	9.4	10.1	9.6	8.3	10.3	10.1	9.4	11.1
Serine	10.8	9.8	8.4	10.8	9.6	8.2	10.5	9.0	7.4
Proline	13.3	12.2	11.5	14.8	14.5	13.6	14.7	14.9	12.0
Alanine	10.8	10.0	9.1	10.1	8.8	7.4	11.2	10.3	9.2
Aspartate	23.6	21.0	17.5	22.1	17.9	13.4	21.3	17.7	13.4
Glutamate	40.4	36.8	32.1	44.9	42.3	38.3	43.6	38.0	32.9
Grower I (18 – 28d)									
Methionine	6.0	5.7	6.0	5.3	5.7	5.8	5.8	5.8	6.6
Cysteine	3.5	3.1	2.8	3.6	3.4	3.0	3.2	3.0	2.5
Met+Cys	9.5	8.8	8.8	8.9	9.1	8.9	9.1	8.8	9.1
Lysine	12.7	11.6	11.5	11.3	11.7	11.1	11.7	11.5	11.9
Threonine	8.3	7.8	7.8	7.9	7.9	7.7	8.1	7.9	8.1
Tryptophan	2.6	2.2	2.0	2.7	2.3	2.0	2.6	2.3	1.9
Arginine	16.3	14.2	12.5	13.5	12.3	11.7	13.3	12.1	12.2
Isoleucine	9.4	8.4	8.2	9.2	8.3	7.8	9.3	8.4	8.3
Leucine	18.3	16.7	15.1	16.6	14.8	12.0	17.8	16.4	14.0
Valine	10.4	9.5	9.6	9.9	9.6	9.4	10.1	9.9	9.8
Histidine	5.9	5.1	4.6	5.3	4.8	3.9	5.0	4.5	4.0
Phenylalanine	11.1	9.9	8.7	10.6	9.5	9.7	10.7	9.6	9.0

Glycine	10.0	8.8	8.6	8.8	8.0	8.8	9.7	8.7	10.4
Serine	10.6	9.5	8.2	10.3	9.1	7.4	10.1	8.8	7.0
Proline	13.1	11.8	10.8	14.4	13.9	19.5	13.8	12.7	11.2
Alanine	10.5	9.7	8.9	9.2	8.3	6.7	10.9	10.3	9.1
Aspartate	22.8	19.8	16.8	19.5	16.4	11.7	20.1	17.1	12.7
Glutamate	39.3	35.5	31.3	45.0	41.9	37.9	42.3	37.9	31.8
Finisher (28 – 18d)									
Methionine	5.7	5.1	6.4	4.7	5.1	5.0	5.6	5.2	6.2
Cysteine	3.3	2.9	2.8	3.5	3.2	3.0	3.2	2.8	2.3
Met+Cys	9.0	8.0	9.3	8.2	8.4	7.9	8.7	7.9	8.5
Lysine	12.0	10.7	12.6	10.4	10.6	9.9	11.3	10.1	11.2
Threonine	8.2	7.2	8.2	7.6	7.4	6.7	8.0	7.3	7.4
Tryptophan	2.5	2.0	2.1	2.5	2.1	1.9	2.6	2.1	1.8
Arginine	16.1	12.6	13.0	12.4	11.0	10.6	12.8	11.0	10.7
Isoleucine	9.1	7.4	8.4	8.5	7.5	7.2	8.8	7.7	7.5
Leucine	17.6	15.3	14.7	15.8	13.3	11.1	17.3	15.8	13.3
Valine	10.1	8.7	9.9	9.3	8.7	8.4	9.6	9.0	8.9
Histidine	5.6	4.6	4.6	5.0	4.3	3.6	4.9	4.1	3.7
Phenylalanine	10.9	8.7	8.5	10.0	8.5	9.0	10.3	8.9	8.5
Glycine	9.8	7.9	8.9	8.3	7.2	7.5	9.4	8.1	9.7
Serine	10.4	8.5	8.4	9.8	8.3	6.8	9.8	8.2	6.4
Proline	12.2	10.7	10.4	14.4	13.2	12.3	13.5	12.5	10.6
Alanine	10.4	9.0	8.8	8.7	7.4	6.0	10.7	10.0	8.8
Aspartate	22.2	17.5	17.0	17.8	13.9	9.7	19.2	15.2	11.0
Glutamate	38.8	31.7	31.1	43.9	39.7	36.7	40.3	35.8	29.2

[1]Control CP
[2]Control CP – 20 g/kg
[3]Control CP – 40 g/kg

Table 6. Growth performance of broiler chickens fed reduced CP diets of varying ingredient combinations at different growth phases

Treatments			Grower I (8-18 d)				Grower II (18-28 d)				Finisher (28 – 38 d)			
Diets	**CP Levels**	**BW @ d8 (kg)**	**BW @ d18 (kg)**	**BWG (kg)**	**FI (kg)**	**FCR**	**BW @ d28 (kg)**	**BWG (kg)**	**FI (kg)**	**FCR**	**BW @ d38 (kg)**	**BWG (kg)**	**FI (kg)**	**FCR**
Corn	Control[1]	0.185	0.746	0.561	0.695^{ab}	1.240^{c}	1.635	0.906	1.271^{b}	1.401	2.738^{abc}	1.140^{abc}	1.829^{bc}	1.607
	Moderate reduction[2]	0.185	0.749	0.563	0.704^{ab}	1.250^{c}	1.630	0.901	1.274^{b}	1.413	2.743^{ab}	1.144^{ab}	1.860^{abc}	1.628
	Strong reduction[3]	0.185	0.715	0.529	0.710^{ab}	1.342^{a}	1.566	0.837	1.288^{b}	1.538	2.685^{bc}	1.086^{bc}	1.861^{abc}	1.713
Wheat & Corn	Control	0.185	0.746	0.560	0.721^{a}	1.286^{bc}	1.611	0.883	1.276^{b}	1.444	2.733^{abc}	1.134^{abc}	1.887^{abc}	1.667
	Moderate reduction	0.185	0.736	0.551	0.701^{ab}	1.271^{bc}	1.611	0.882	1.309^{ab}	1.485	2.714^{abc}	1.115^{abc}	1.946^{a}	1.746
	Strong reduction	0.185	0.704	0.519	0.680^{b}	1.310^{ab}	1.575	0.846	1.348^{a}	1.596	2.642^{cd}	1.043^{cd}	1.901^{ab}	1.822
Sorghum & Wheat	Control	0.185	0.727	0.543	0.684^{ab}	1.262^{bc}	1.594	0.866	1.269^{b}	1.467	2.772^{a}	1.174^{a}	1.926^{a}	1.642
	Moderate reduction	0.185	0.735	0.550	0.705^{ab}	1.283^{bc}	1.601	0.872	1.289^{ab}	1.478	2.698^{bc}	1.099^{bc}	1.900^{abc}	1.730
	Strong reduction	0.185	0.699	0.514	0.691^{ab}	1.345^{a}	1.541	0.812	1.276^{b}	1.573	2.603^{d}	1.005^{d}	1.806^{c}	1.788
SEM		0.0001	0.0027	0.0027	0.0029	0.0058	0.0072	0.0052	0.0045	0.0071	0.0136	0.0081	0.0083	0.0093
Main effects														
Diets														
	Corn	0.185	0.736^{a}	0.551^{a}	0.703	1.277	1.610^{a}	0.882^{a}	1.277	1.451^{b}	2.722	1.123	1.850	1.649^{b}
	Wheat & Corn	0.185	0.729^{ab}	0.543^{ab}	0.700	1.289	1.599^{ab}	0.870^{ab}	1.310	1.508^{a}	2.696	1.098	1.912	1.745^{a}
	Sorghum & Wheat	0.185	0.721^{b}	0.535^{b}	0.693	1.295	1.579^{b}	0.850^{b}	1.278	1.506^{a}	2.691	1.092	1.877	1.723^{a}
CP Levels														
	Control	0.185	0.740^{a}	0.555^{a}	0.700	1.261	1.613^{a}	0.885^{a}	1.272	1.437^{b}	2.747	1.149	1.881	1.639^{c}
	Moderate reduction	0.185	0.740^{a}	0.555^{a}	0.703	1.268	1.614^{a}	0.885^{a}	1.290	1.458^{b}	2.718	1.120	1.902	1.701^{b}
	Strong reduction	0.185	0.706^{b}	0.521^{b}	0.694	1.332	1.560^{b}	0.832^{b}	1.304	1.569^{a}	2.643	1.045	1.856	1.777^{a}
p-Value														
	Diets	0.9701	0.0019	0.0023	0.3052	0.1337	0.0091	0.0091	0.0006	<0.0001	0.0923	0.0923	0.0009	<0.0001
	CP Levels	0.7657	<0.0001	<0.0001	0.3219	<0.0001	0.0010	0.0010	0.0457	<0.0001	<0.0001	<0.0001	0.0989	<0.0001
	Diet* CP Levels	0.8911	0.5655	0.6041	0.0072	0.0280	0.6316	0.6316	0.0474	0.3538	0.0133	0.0133	0.0009	0.2562

[abcd]Means with different superscripts across the columns are significantly ($p<0.05$) different.

BW, Body weight; **WG**, Weight gain; **FI**, Feed intake; **FCR**, Feed Conversion Ratio

[1] Grower I = 230 g/kg CP; Grower II = 215 g/kg CP; Finisher: 205 g/kg CP

[2] Grower I = 210 g/kg CP; Grower II = 195 g/kg CP; Finisher: 185 g/kg CP

[3] Grower I = 190 g/kg CP; Grower II = 175 g/kg CP; Finisher: 165 g/kg CP

Table 7. Cumulative growth performance of broiler chickens fed reduced CP diets of varying ingredient combinations (8 – 38d)

Treatments		BW @ d8 (kg)	BW @ d38 (kg)	BWG (kg)	FI (kg)	FCR
Diets	**CP Levels**					
Corn	Control[1]	0.185	2.799	2.613	3.794	1.452
	Moderate reduction[2]	0.185	2.802	2.617	3.852	1.472
	Strong reduction[3]	0.185	2.572	2.447	3.811	1.557
Wheat & Corn	Control	0.185	2.762	2.577	3.897	1.513
	Moderate reduction	0.185	2.734	2.549	3.937	1.545
	Strong reduction	0.185	2.573	2.388	3.855	1.615
Sorghum & Wheat	Control	0.185	2.779	2.593	3.851	1.485
	Moderate reduction	0.185	2.708	2.523	3.879	1.538
	Strong reduction	0.185	2.509	2.324	3.708	1.596
SEM		0.0001	0.0136	0.0136	0.0118	0.0068
Main effects						
Diets						
	Corn	0.185	2.744[a]	2.559[a]	3.819[b]	1.494[c]
	Wheat & Corn	0.185	2.690[b]	2.505[b]	3.896[a]	1.557[a]
	Sorghum & Wheat	0.185	2.665[b]	2.480[b]	3.813[b]	1.540[b]
CP Levels						
	Control	0.185	2.780[a]	2.595[a]	3.847[ab]	1.483[c]
	Moderate reduction	0.185	2.748[a]	2.563[a]	3.889[a]	1.518[b]
	Strong reduction	0.185	2.572[b]	2.386[b]	3.791[b]	1.589[a]
p-Value						
	Diets	0.9701	<0.0001	<0.0001	0.0014	<0.0001
	CP Levels	0.7657	<0.0001	<0.0001	0.0006	<0.0001
	Diet* CP Levels	0.8911	0.1416	0.1433	0.0810	0.2860

[abc]Means with different superscripts across the columns are significantly ($p<0.05$) different.
BW, Body weight; **WG**, Weight gain; **FI**, Feed intake; **FCR**, Feed Conversion Ratio
[1]Control CP
[2]Control CP – 20 g/kg
[3]Control CP – 40 g/kg

Table 8. Total body composition of broiler chickens fed reduced CP diets of varying ingredient combinations

Treatments		**Intake**		**Deposition**		**Ratio**	
Diets	**CP Levels**	**Protein (gDM/b*d)**	**Fat (gDM/b*d)**	**Pd (gDM/b*d)**	**Fd (gDM/b*d)**	**kP (g/g)**	**kF (g/g)**
Corn	Control[1]	26.14^{a}	8.74^{b}	12.83	7.70^{d}	0.49^{b}	0.88^{d}
	Moderate reduction[2]	23.17^{b}	7.58^{d}	13.41	8.97^{abc}	0.58^{a}	1.19^{c}
	Strong reduction[3]	21.63^{c}	6.33^{e}	12.77	9.04^{abc}	0.59^{a}	1.42^{b}
Wheat & Corn	Control	25.52^{a}	8.39^{c}	13.56	8.70^{bcd}	0.54^{ab}	1.07^{c}
	Moderate reduction	23.53^{b}	8.42^{c}	12.77	9.45^{ab}	0.54^{ab}	1.12^{c}
	Strong reduction	20.95^{c}	6.38^{e}	11.95	9.05^{abc}	0.57^{a}	1.41^{b}
Sorghum & Wheat	Control	25.66^{a}	9.82^{a}	13.11	7.92^{cd}	0.51^{b}	0.80^{d}
	Moderate reduction	23.05^{b}	8.18^{c}	12.55	9.52^{ab}	0.55^{ab}	1.18^{c}
	Strong reduction	19.82^{d}	6.05^{f}	11.78	10.05^{a}	0.59^{a}	1.66^{a}
SEM		0.2531	0.1443	0.0001	0.0001	0.0052	0.0276
Main effects							
Diets							
	Corn	23.64	7.55	13.00	8.57	0.55	1.64
	Wheat & Corn	23.34	7.73	12.76	9.07	0.55	1.20
	Sorghum & Wheat	22.84	8.02	12.48	9.17	0.55	1.21
CP Levels							
	Control	25.78	8.98	13.16^{a}	8.11	0.51	0.92
	Moderate reduction	23.25	8.06	12.91^{a}	9.32	0.56	1.16
	Strong reduction	20.80	6.26	12.17^{b}	9.38	0.58	1.50
p-Value							
	Diets	<0.0001	<0.0001	0.0918	0.0173	0.9138	0.2636
	CP Levels	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
	Diet * CP Levels	<0.0001	<0.0001	0.0594	0.0258	0.0108	<0.0001

^{abcd}Means with different superscripts across the columns are significantly ($p<0.05$) different.

[1]Control CP, [2]Control CP – 20 g/kg, [3]Control CP – 40 g/kg

Pd – Protein deposition; **Fd** – Fat deposition; **kP** – Ratio of protein deposition to intake; **kF** – Ratio of fat deposition to intake

$Pd = \frac{BW * \%CP_{DM}}{number\ of\ days};\ Fd = \frac{BW * \%fat_{DM}}{number\ of\ days};\ Protein\ intake = Pd * FI;\ Fat\ intake = Fd * FI;\ kP = \frac{Pd}{Protein\ intake};\ kF = \frac{Fd}{Fat\ intake}$

Table 9. Nitrogen excretion of broiler chickens fed reduced CP diets of varying ingredient combinations

Treatments		**N_i (g/b*d)**	**N_R (g/b*d)**	**AN_e (g/b*d)**	**AN_eC (g/g)**
Diets	**CP Levels**				
Corn	Control[1]	4.17[a]	2.05	2.12	0.51
	Moderate reduction[2]	3.70[b]	2.15	1.56	0.42
	Strong reduction[3]	3.47[c]	2.04	1.43	0.41
Wheat & Corn	Control	4.16[a]	2.19	1.99	0.48
	Moderate reduction	3.77[b]	2.04	1.72	0.46
	Strong reduction	3.37[c]	1.87	1.49	0.44
Sorghum & Wheat	Control	4.12[a]	2.10	2.02	0.49
	Moderate reduction	3.67[b]	2.01	1.67	0.45
	Strong reduction	3.17[d]	1.88	1.29	0.41
SEM		0.0459	0.0227	0.0416	
Main effects					
Diets					
	Corn	3.78	2.08	1.70	0.45
	Wheat & Corn	3.77	2.03	1.73	0.46
	Sorghum & Wheat	3.65	2.00	1.66	0.45
CP Levels					
	Control	4.15	2.11[a]	2.04[a]	0.49[a]
	Moderate reduction	3.71	2.07[a]	1.65[b]	0.44[b]
	Strong reduction	3.34	1.93[b]	1.40[c]	0.42[b]
p-Value					
	Diets	<0.0001	0.2394	0.3586	0.6489
	CP Levels	<0.0001	0.0018	<0.0001	<0.0001
	Diet* CP Levels	<0.0001	0.1606	0.0885	0.2085

[abcd]Means with different superscripts across the columns are significantly ($p<0.05$) different.

[1]Control CP

[2]Control CP – 20 g/kg

[3]Control CP – 40 g/kg

N_i – Nitrogen intake; **N_R** – Nitrogen retained; **AN_e** – Apparent nitrogen excretion; **AN_eC** - Apparent nitrogen excretion coefficient

Table 10. Carcass traits of broiler chickens fed reduced CP diets of varying ingredient combinations

Treatments		Fasted body weight (g)	Carcass weight (g)	Relative fasted body weight, %			Relative carcass weight, %
				Carcass yield	Abdominal fat	Liver	Breast yield
Diets	**CP Levels**						
Corn	Control[1]	2577.50[a]	1949.67[ab]	75.64	0.83[d]	1.79	36.36[abc]
	Moderate reduction[2]	2599.78[a]	1977.74[a]	76.07	0.91[cd]	1.83	37.29[a]
	Strong reduction[3]	2457.17[c]	1853.78[c]	75.44	1.24[ab]	2.05	36.23[abc]
Wheat & Corn	Control	2557.29[ab]	1930.33[ab]	75.48	0.80[d]	1.87	35.22[bcd]
	Moderate reduction	2528.26[abc]	1910.35[abc]	75.55	1.14[b]	2.00	36.79[ab]
	Strong reduction	2376.09[d]	1773.13[d]	74.60	1.13[bc]	2.13	35.19[cd]
Sorghum & Wheat	Control	2577.50[a]	1946.38[ab]	75.52	0.88[d]	1.87	36.28[abc]
	Moderate reduction	2491.67[bc]	1880.57[bc]	75.46	0.92[cd]	1.92	36.00[abc]
	Strong reduction	2344.17[d]	1742.54[d]	74.33	1.36[a]	2.03	34.02[d]
SEM		8.4504	7.5160	0.0976	0.0202	0.0150	0.1325
Main effects							
Diets							
	Corn	2544.82	1927.06	75.71[a]	1.00	1.89[b]	36.63
	Wheat & Corn	2487.21	1871.27	75.21[ab]	1.02	2.00[a]	35.74
	Sorghum & Wheat	2471.11	1856.50	75.12[b]	1.05	1.94[ab]	35.43
CP Levels							
	Control	2570.76	1941.13	75.51[a]	0.83	1.85[b]	35.95
	Moderate reduction	2539.90	1922.89	75.70[a]	0.99	1.92[b]	36.70
	Strong reduction	2392.48	1789.92	74.81[b]	1.25	2.07[a]	35.15
p-Value							
	Diets	<0.0001	<0.0001	0.0183	0.3659	0.0032	0.0001
	CP Levels	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
	Diet* CP Levels	0.0143	0.0103	0.4996	<0.0001	0.4731	0.0083

[abcd]Means with different superscripts across the columns are significantly ($p<0.05$) different.
[1]Control CP
[2]Control CP – 20 g/kg
[3]Control CP – 40 g/kg

Table 11. Apparent ileal nutrient digestibility coefficients of broiler chickens fed reduced CP diets of varying ingredient combinations at d 40

Treatments		**Digestibility coefficients**		
Diets	**CP Levels**	**CP**	**Energy**	**DE**
Corn	Control[1]	0.86	0.82	3142
	Moderate reduction[2]	0.88	0.85	3106
	Strong reduction[3]	0.88	0.85	3079
Wheat & Corn	Control	0.86	0.79	2945
	Moderate reduction	0.85	0.77	2902
	Strong reduction	0.89	0.79	2872
Sorghum & Wheat	Control	0.83	0.78	3071
	Moderate reduction	0.85	0.81	3015
	Strong reduction	0.86	0.82	2976
SEM		0.0037	0.0054	19.4747
Main effects				
Diets				
	Corn	0.88[a]	0.84[a]	3109[a]
	Wheat & Corn	0.87[a]	0.78[b]	2907[b]
	Sorghum & Wheat	0.85[b]	0.80[b]	3021[a]
CP Levels				
	Control	0.85[b]	0.80	3053
	Moderate reduction	0.86[b]	0.81	3008
	Strong reduction	0.88[a]	0.82	2976
p-Value				
	Diets	0.0007	<0.0001	0.0001
	CP Levels	0.0009	0.0870	0.1869
	Diet* CP Levels	0.2256	0.3993	0.9987

[abcd]Means with different superscripts across the columns are significantly ($p<0.05$) different.
[1]Control CP
[2]Control CP – 20 g/kg
[3]Control CP – 40 g/kg
CP– Crude protein; **DE** – Digestible energy

Table 12.1 Apparent ileal amino acid digestibility coefficients of broiler chickens fed reduced CP diets of varying ingredient combinations at d 40

Treatments		**Methionine**	**Cysteine**	**Lysine**	**Threonine**	**Arginine**	**Isoleucine**	**Leucine**	**Valine**
Diets	**CP Levels**								
Corn	Control[1]	0.96	0.82[ab]	0.93	0.86[abc]	0.95	0.90[abc]	0.90	0.89[abc]
	Moderate reduction[2]	0.97	0.84[ab]	0.94	0.88[abc]	0.95	0.91[ab]	0.91	0.90[ab]
	Strong reduction[3]	0.97	0.83[ab]	0.95	0.89[a]	0.95	0.93[a]	0.91	0.92[a]
Wheat & Corn	Control	0.94	0.82[ab]	0.92	0.85[bc]	0.92	0.90[bcd]	0.88	0.88[bcd]
	Moderate reduction	0.94	0.82[ab]	0.91	0.83[cd]	0.91	0.87[de]	0.85	0.86[de]
	Strong reduction	0.96	0.87[a]	0.94	0.88[ab]	0.93	0.92[ab]	0.89	0.91[ab]
Sorghum & Wheat	Control	0.93	0.80[b]	0.91	0.80[d]	0.92	0.85[e]	0.84	0.83[e]
	Moderate reduction	0.95	0.81[b]	0.92	0.85[bc]	0.92	0.88[cd]	0.86	0.87[cd]
	Strong reduction	0.96	0.79[b]	0.94	0.87[abc]	0.93	0.90[bcd]	0.86	0.89[bcd]
SEM		0.0018	0.0046	0.0025	0.0040	0.0024	0.0035	0.0041	0.0039
Main effects									
Diets									
	Corn	0.97[a]	0.83	0.94[a]	0.88	0.95[a]	0.92	0.91[a]	0.91
	Wheat & Corn	0.95[b]	0.84	0.92[b]	0.85	0.92[b]	0.90	0.87[b]	0.88
	Sorghum & Wheat	0.95[b]	0.80	0.93[b]	0.84	0.92[b]	0.87	0.85[c]	0.86
CP Levels									
	Control	0.95[b]	0.82	0.92[b]	0.84	0.93[b]	0.88	0.87	0.87
	Moderate reduction	0.95[b]	0.84	0.92[b]	0.85	0.93[b]	0.89	0.87	0.87
	Strong reduction	0.97[a]	0.83	0.95[a]	0.88	0.94[a]	0.92	0.89	0.90
p-Value									
	Diets	<0.0001	0.0018	0.0012	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	CP Levels	<0.0001	0.2772	<0.0001	<0.0001	0.0034	<0.0001	0.1793	<0.0001
	Diet* CP Levels	0.0774	0.0441	0.3679	0.0393	0.3398	0.0024	0.0758	0.0015

[abcde]Means with different superscripts across the columns are significantly ($p<0.05$) different.

[1]Control CP

[2]Control CP – 20 g/kg

[3]Control CP – 40 g/kg

Table 12.2 Apparent ileal amino acid digestibility coefficients of broiler chickens fed reduced CP diets of varying ingredient combinations at d 40

Treatments		**Histidine**	**Phenylalanine**	**Glycine**	**Serine**	**Proline**	**Alanine**	**Aspartate**	**Glutamate**
Diets	**CP Levels**								
Corn	Control[1]	0.90	0.92[ab]	0.85	0.89	0.87[bc]	0.90[ab]	0.90	0.92[bcd]
	Moderate reduction[2]	0.91	0.92[ab]	0.85	0.89	0.89[ab]	091[a]	0.90	0.93[abc]
	Strong reduction[3]	0.91	0.93[ab]	0.87	0.89	0.88[bc]	0.91[a]	0.90	0.93[abc]
Wheat & Corn	Control	0.89	0.90[bc]	0.85	0.88	0.89[ab]	0.87[bc]	0.87	0.92[bcd]
	Moderate reduction	0.87	0.88[cd]	0.81	0.86	0.88[abc]	0.83[c]	0.84	0.91[bcd]
	Strong reduction	0.90	0.93[a]	0.89	0.88	0.92[a]	0.87[bc]	0.86	0.95[a]
Sorghum & Wheat	Control	0.87	0.86[d]	0.83	0.85	0.85[c]	0.83[c]	0.85	0.88[e]
	Moderate reduction	0.87	0.89[cd]	0.84	0.86	0.86[bc]	0.86[c]	0.86	0.90[cd]
	Strong reduction	0.87	0.90[bc]	0.89	0.84	0.84[c]	0.85[c]	0.85	0.90[d]
SEM		0.0032	0.0033	0.0041	0.0037	0.0038	0.0048	0.0041	0.0029
Main effects									
Diets									
	Corn	0.90[a]	0.92	0.86	0.89[a]	0.88	0.91	0.90[a]	0.93
	Wheat & Corn	0.89[b]	0.91	0.85	0.87[b]	0.90	0.86	0.86[b]	0.93
	Sorghum & Wheat	0.87[c]	0.89	0.85	0.85[c]	0.85	0.85	0.85[b]	0.90
CP Levels									
	Control	0.89	0.90	0.84[b]	0.88	0.87	0.87	0.87	0.91
	Moderate reduction	0.88	0.90	0.84[b]	0.87	0.88	0.87	0.86	0.91
	Strong reduction	0.89	0.92	0.88[a]	0.87	0.88	0.88	0.87	0.93
p-Value									
	Diets	<0.0001	<0.0001	0.5950	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
	CP Levels	0.2850	<0.0001	<0.0001	0.6715	0.2599	0.1514	0.4113	0.0001
	Diet* CP Levels	0.1390	0.0003	0.0755	0.2088	0.0119	0.0499	0.2937	0.0066

[abcd]Means with different superscripts across the columns are significantly ($p<0.05$) different.

[1]Control CP

[2]Control CP – 20 g/kg

[3]Control CP – 40 g/kg

CHAPTER 3 – Implications

The rapid growth of broiler chickens justifies their need for high dietary protein compared to other livestock, except turkey and fish. Metabolism of this high protein by the chickens yields high nitrogenous waste, which is lost into the environment as ammonia, contributing on one hand, to global warming, and also have the tendency to worsen the birds' welfare, which might reduce the meat quality of the birds. Furthermore, soybean meal is the most used protein source in broiler diets. The need for environmental sustainability, improved broiler welfare, reduction of overdependence on soybean meal heightens the importance of the need for reduced protein research (with different commonly used feed ingredient combinations) in broilers.

This study showed that if all necessary amino acids are balanced in broiler diets, their dietary protein requirement can be reduced up to 20 g/kg, irrespective of whether corn, wheat-corn, or sorghum wheat is used as energy source, at all growth phases without negatively impacting their productivity, yet, reducing the nitrogen excreted into the environment. It was as well deduced that feed conversion ratio alone may not be enough metric to measure productivity when feeding reduced protein diet to broilers. On average, when reducing dietary protein of broiler chickens up to 20 g/kg, soybean meal in the diet can be reduced up to 21%, 27%, and 23%, when corn, wheat-corn, and sorghum-wheat diets, respectively, are fed to the birds.

From a scientific point of view, the study confirmed that the increased starch as a result of reduced protein diet causes increased fat deposition in the broiler chickens. Since most feed formulations are done using the apparent metabolizable energy which does not take heat increment into consideration, it is hypothesized that formulating using the net energy or effective energy system may cancel out the impact of heat increment on the diet formulation to an extent, thereby reducing incidence of increased fat deposition.