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CÂMPUS DE JABOTICABAL

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

Damilola Uthman Kareem

Animal Scientist

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UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CÂMPUS DE JABOTICABAL

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Discente: Damilola Uthman Kareem

Orientadora: Profª. Drª. Nilva Kazue Sakomura

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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

AUTOR: DAMILOLA UTHMAN KAREEM
ORIENTADORA: NILVA KAZUE SAKOMURA

Aprovado como parte das exigências para obtenção do Título de Mestre em Ciência Animal, área: Nutrição Animal pela Comissão Examinadora:

Profa. Dra. NILVA KAZUE SAKOMURA (Participaçao Virtual)
Departamento de Zootecnia / FCAV UNESP Jaboticabal

Pós-doutorando MATHEUS DE PAULA REIS (Participaçao Virtual)
Departamento de Zootecnia / FCAV UNESP Jaboticabal

Dr. GABRIEL DA SILVA VIANA (Participaçao Virtual)
Natural Resources Institute Finland/LUKE / Jokioinen/Finlândia

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 Prof[a]. Dr[a]. 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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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

Faculdade de Ciências Agrárias e Veterinárias
Via de Acesso Prof. Paulo Donato Castellane, s/n CEP 14884-900 - Jaboticabal - SP - Brasil
Tel. 16 3209-7100 - www.fcav.unesp.br

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.

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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