UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS
CÂMPUS DE JABOTICABAL

ASSESSING ENERGY METABOLISM IN BROILER CHICKENS: METHODOLOGY FOR EVALUATION AND DETERMINING THE ENERGY CONTENT OF FEED INGREDIENTS

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Animal Science

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IMPACTO POTENCIAL DESTA PESQUISA

A produção avícola no Brasil representa uma das principais atividades econômicas no setor agropecuário. Os resultados desta tese representam um avanço para a implementação de um sistema de energia líquida na formulação de rações, permitindo uma estimativa mais precisa do valor energético dos alimentos e a otimização dos custos de alimentação.

POTENTIAL IMPACT OF THIS RESEARCH

Poultry production in Brazil represents one of the main economic activities in the agricultural sector. The results of this thesis represent a significant advancement towards implementing a net energy system in feed formulation, enabling a more accurate estimation of the energy value of feed ingredients and the optimization of feeding cost.

UNIVERSIDADE ESTADUAL PAULISTA

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CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: ASSESSING ENERGY METABOLISM IN BROILER CHICKENS: METHODOLOGY FOR EVALUATION AND DETERMINING THE ENERGY CONTENT OF FEED INGREDIENTS

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Jaboticabal, 11 de março de 2024

CURRICULAR INFORMATION

RONY RIVEROS LIZANA – Natural from Peru, born on September 7th, 1993. Started his undergraduate studies in Animal Husbandry in 2011 at the National Agrarian University “La Molina”– Lima, Peru, obtaining the bachelor’s degree in animal Husbandry in 2016. On the last period of undergrad, started as researcher assistantship collaborating in human nutrition researcher in a project of National Council for Science and Technology (from acronym CONCYTEC, Lima, Peru). During the period 2016-2017 he was recruited by the local company as Jr. Research working elaborating and supervising experiments in poultry nutrition. In 2018, he moved to Brazil to enhance his knowledge, applying and being approved in the top position on the non-ruminant nutrition area to the master’s degree in the graduate program in the Sao Paulo State University “Júlio de Mesquita Filho” (FCAV–UNESP), Jaboticabal Campus, under the supervision of Prof. Dr. Nilva Kazue Sakomura and co-supervision by Dr. Jaap van Milgen from INRAe (France). Throughout his master’s studies, he actively contributed to the development and execution of the thematic project “Net Energy for Poultry” coordinated by Prof. Dr. Nilva Sakomura, funded by the São Paulo Research Foundation (FAPESP). In March 2020, he completed his master’s degree and was subsequently admitted to the Ph.D. program, ranking first in the non-ruminant area, under the supervision of Prof. Dr. Nilva Sakomura. During his Ph.D., he continued to be involved in the thematic project, conducting his doctoral research on the development of equations for net energy in broilers, along with his collaboration on other projects involving calcium and phosphorus modeling for poultry and poultry growth modeling.

To my parents, who anyway, are responsible for myself be here and who I am today.

To my brother, for his guidance, from our shared childhood.

To my dream, that just for me *“make sence”*.

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SUMMARY

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de Jaboticabal

CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

C E R T I F I C A D O

Certificamos que o projeto de pesquisa intitulado **"Determinação da energia líquida de ingredientes para frangos de corte"**, protocolo nº 5401/20, 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 10 de dezembro de 2020.

Vigência do Projeto	03/10/2021 a 17/03/2022
Espécie / Linhagem	*Gallus gallus domesticus* / Cobb 500
Nº de animais	1400
Peso / Idade	45 g / 1 dia
Sexo	Machos
Origem	Incubatório Pluma, Descalvado - SP

Jaboticabal, 10 de dezembro de 2020.

Fabiana Pilarski

Profa. Dra. Fabiana Pilarski
Coordenadora – CEUA

Faculdade de Ciências Agrárias e Veterinárias
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ASSESSING ENERGY METABOLISM IN BROILER CHICKENS: METHODOLOGY FOR EVALUATION AND DETERMINING THE ENERGY CONTENT OF FEED INGREDIENTS

RESUMO – Este trabalho visa estabelecer o sistema de energia líquida (EL) para frangos de corte, elucidando o metabolismo energético das aves e os métodos metodológicos empregados na investigação desse metabolismo. Para atingir esse objetivo, realizou-se uma revisão bibliográfica, ensaios de validação do sistema de calorimetria indireta (CI) e ensaios biológicos para determinar o valor de EL dos alimentos para frangos de corte. O sistema de EL para esses animais tem sido pouco explorado, considerando que sua aplicação na formulação de rações comerciais permanece limitada. Nesse contexto, efetuou-se uma revisão bibliográfica abrangente, com foco nos princípios conceituais, na evolução dos estudos sobre metabolismo energético em aves e nas diversas metodologias utilizadas para a sua avaliação. Os ensaios experimentais foram realizados utilizando-se seis câmaras de respirometria, fundamentadas no sistema de CI de circuito aberto com pressão negativa, equipadas com gaiolas metabólicas, bebedouros e comedouros do tipo calha, configurados para acomodar grupos de frangos de corte. O sistema de fluxo foi monitorado por uma bomba de pressão negativa com fluxômetro integrado para controle do fluxo. Os gases foram mensurados por um analisador paramagnético de O_2 e um analisador infravermelho de CO_2, integrando um conjunto de componentes característicos da linha Classic Line da Sable System. Adicionalmente, conduziu-se um ensaio para implementar e avaliar o sistema de CI por meio de procedimentos estatísticos e simulações, considerando a dinâmica dos gases atmosféricos. Desse ensaio, desenvolveu-se uma planilha em MS Excel para automatizar os cálculos da produção de calor a partir do volume de consumo de O_2 e produção de CO_2, permitindo verificar a taxa de recuperação dos gases e, consequentemente, a viabilidade do sistema de CI. Após a validação do sistema de CI, realizou-se um ensaio com frangos de corte de 15 a 21 dias de idade, objetivando desenvolver equações de predição de EL dos ingredientes, utilizando 48 dietas formuladas com ingredientes tradicionais e não tradicionais, visando uma ampla variação na composição nutricional. As medições a mensuração da produção de calor e coleta de excretas, para determinação da EMA, EMAn e EL. O desenvolvimento das equações de predição de EL baseando-se na composição nutricional através de análises de regressão múltipla. A avaliação do valor energético de fontes de óleo e gordura seguiu o protocolo do experimento anterior, determinando o valor energético (AME e EL) do óleo de soja e gordura de aves. Por fim, propôs-se um modelo teórico mecanicista para estimar o valor de EL dos alimentos, considerando a utilização dos nutrientes e o metabolismo animal. Os resultados obtidos sublinham a relevância do estudo do metabolismo energético, considerando os procedimentos metodológicos e a determinação do valor de EL dos ingredientes para frangos de corte, demonstrando sua aplicabilidade na produção comercial de aves.

Palavras-chave: Calorimetria indireta, frangos de corte, metabolismo energético, utilização de energia

ABSTRACT – This work aims to establish the net energy (NE) system for broiler chickens, elucidating the energy metabolism of the birds and the methodological approaches used in the investigation of this metabolism. To achieve this goal, a literature review was conducted, along with validation assays of the indirect calorimetry (IC) system and biological assays to determine the NE value of feed for broiler chickens. The NE system for these animals has been sparsely explored, given that its application in the formulation of commercial feeds remains limited. In this context, a comprehensive literature review was conducted, focusing on conceptual principles, the evolution of studies on avian energy metabolism, and the various methodologies used for its evaluation. The experimental assays were conducted using six respirometry chambers, based on the open-circuit IC system with negative pressure, equipped with metabolic cages, waterers, and trough feeders, configured to accommodate groups of broiler chickens. The flow system was monitored by a negative pressure pump with an integrated flowmeter for flow control. Gases were measured by a paramagnetic O_2 analyzer and an infrared CO_2 analyzer, integrating a set of components characteristic of the Sable System Classic Line. Additionally, an assay was conducted to implement and evaluate the IC system through statistical procedures and simulations, considering the dynamics of atmospheric gases. From this assay, a spreadsheet in MS Excel was developed to automate the calculations of heat production from the volume of O_2 consumption and CO_2 production, allowing the verification of gas recovery rate and, consequently, the viability of the IC system. Following the validation of the IC system, an assay was conducted with broiler chickens aged 15 to 21 days, aiming to develop prediction equations for the NE of ingredients, using 48 diets formulated with traditional and non-traditional ingredients, aiming for a wide variation in nutritional composition. Measurements included the measurement of heat production and collection of excreta, for the determination of apparent metabolizable energy (AME), nitrogen-corrected AME (AMEn), and NE. The development of NE prediction equations was based on nutritional composition through multiple regression analyses. The evaluation of the energy value of oil and fat sources followed the protocol of the previous experiment, determining the energy value (AME and NE) of soy oil and poultry fat. Finally, a mechanistic theoretical model was proposed to estimate the NE value of feed, considering nutrient utilization and animal metabolism. The obtained results underline the relevance of studying energy metabolism, considering the methodological procedures and the determination of the NE value of ingredients for broiler chickens, demonstrating its applicability in the commercial production of poultry.

Keywords: Broiler chickens, energy metabolism, energy utilization, indirect calorimetry.

CHAPTER 1 – General considerations

GENERAL CONSIDERATIONS

Introduction

Energy is one of the most important components in poultry diets, essential to broiler chickens' growth and development. Additionally, dietary energy content is known as the chief driver of feed intake regulation, as broiler chickens intake enough feed to meet their energy requirements for maintenance and growth (Emmans, 1994; Hughes, 2008; Lopez and Leeson, 2008). Consequently, other essential nutrients, chiefly amino acids, must be proportionally adjusted in relation to energy. Modern broiler chickens consume approximately 10% of their body weight in dry matter bases (Nascimento et al., 2020). In this sense, these birds demonstrate high growth rates even when exposed to significant variations in dietary energy concentrations (Zuidhof et al., 2014). However, it is undeniable that dietary energy influences on the body composition (Lopez and Leeson, 2008).

The complex relationship between energy and nutrients, their mechanism involved in feed intake regulation, and how they influence body composition make it an important topic to understand how animals use energy. The intricate understanding of energy metabolism in poultry chickens has been a continuous and enduring subject of interest since the early days of modern poultry science (NRC, 1994). In the early 20th century, researchers and nutritionists recognized the importance of achieving optimal energy balance in poultry diets to enhance growth, production, and overall performance (Hurwitz et al., 1978). Meanwhile, the energy utilization of birds and understanding how the animal allocates energy under different conditions are currently being studied, along with a more accurate determination of the energy values of feeds. This interest from researchers was favored by technological advancements, efficient dissemination, and ease of sharing results, as well as more fluid communication among researchers, promoting the standardization of procedures and methodological protocols that reduce the variability of results and their discrepancies. Methodological tools and their proper application are crucial in studies of energetic metabolism and any biological assay.

Regarding studies about energetic metabolism through biological assays, two components must be considered: the animal ("machine") and the feed ("fuel"). The observation of both factors that induce (the feed) and express (the animal) a response (variations on the metabolism) should be considered simultaneously to elucidate the questions proposed in each study. This introspection about the energy metabolism evaluation in poultry and their corresponding interpretations resulted from discussions inspired by Dr. Jaap van Milgen lectures, so both this review and the manuscripts presented here attempt, in part, to propose results based on these interpretations.

In practical terms, feed evaluations are the main objective of nutritionists due to the feed cost involved in the animal protein production industry. The primary energy sources in poultry diets are cereal grains, generally complemented by vegetable oils derived from seeds. Depending on geographical regions, corn may dominate practical diets in Brazil and the United States, while wheat may prevail in Europe and Australia (Ravindran and Brair, 1991). Other ingredients like triticale, barley, and sorghum are sporadic and contingent upon availability (Hughes, 2003). It is worth noting that the available energy content of these ingredients varies significantly based on their physicochemical composition (Hughes and Choct, 1999). In this way, it is important to explore novel systems to evaluate energy accurately.

In this sense, this work was developed to explore the principal conceptual bases involved in the energy metabolism of broiler chickens and the methodological tools used for energy metabolism studies and reach a domine of the topic to develop the net energy system to be applied in broiler chickens.

Literature review

Energy utilization

The energy requirement of broiler chickens refers to the amount of dietary energy needed to support various physiological processes, including maintenance, growth, reproduction, and activity (Sakomura and Rostagno, 2017).

Energy requirements are influenced by body weight, age, sex, genetic background, environmental conditions, and production goals (Sakomura et al., 2004). For example, broiler chickens have different energy requirements at different stages of growth. The early growth phase requires higher energy levels for rapid growth, while the later stages focus on maintaining body weight and promoting efficient feed conversion (Uftab, 2019). The birds use dietary energy between the more representative fractions for maintenance, growth, activity, thermoregulation, and development of feathers (Riveros et al., 2023).

The energy in broiler diets is primarily used for maintenance, growth, and other metabolic functions. Maintenance energy is the amount required to keep the bird alive and functioning at a basic level, including keeping its body temperature regulated and organs functioning (Kilbles and Brody, 1944; Noblet et al., 2015). Any energy intake beyond maintenance needs contributes to growth, including the development of muscle (meat) and, to a lesser extent, fat (Kuenzel, 1977; Rabello et al., 2014). As fast-growing birds, broilers have high metabolic and tissue synthesis rates, necessitating high energy density, usually from the dietary carbohydrates and fats (Cerrate et al., 2019; Martinez et al., 2023). For that, the body composition is influenced by the nutritional composition of the diet and the sources of energy (Cerrate et al., 2019). Additionally, many other factors, like environmental conditions, health status, etc., are critical factors that influence energy utilization and, consequently, their overall production efficiency (Sakomura et al., 2004).

For all of that, understanding energy utilization helps to optimize feed formulation and management practices. Modern breeding practices have significantly improved the feed conversion ratio (FCR), which measures the efficiency with which birds convert feed into body mass or contrasts how dietary energy is converted into body-retained energy, reflecting as the efficiency of energy utilization. A lower FCR indicates more efficient energy utilization, a key goal in broiler production to reduce costs and improve sustainability (de Groot., 1980). Under this proposition, it can be interpreted that the expression of an energy requirement and supply of the same amount of energy on the feed can be reflected directly in improving FCR.

Maintenance

A portion of the dietary energy is utilized for maintenance, which includes basal metabolic rate (BMR) and is represented by the energy expended (or fasting heat production) when the bird is at rest and in a thermoneutral environment. This energy is the minimum amount sufficient to support the organ function, respiration, digestion, and other essential metabolic processes (Noblet et al., 2015; Labusiere et al., 2017; Liu et al., 2014; Martinez et al., 2023)

Factors such as body weight, age, sex, and genetic background influence the maintenance energy requirements of broiler chickens (Noblet et al., 2015).

Growth

The remaining energy beyond maintenance requirements is allocated for growth, which includes muscle deposition, skeletal development, and other anabolic processes (Liu et al., 2014; Caldas et al., 2023). Protein synthesis is an energy-demanding process, and broiler chickens have a high capacity for muscle growth (Vignale et al., 2020). Consequently, adequate energy availability is crucial to support rapid growth rates and achieve optimal body composition. Genetic factors, dietary nutrient composition, and environmental conditions can influence energy retention in both protein and fat tissues (Sakomura et al., 2004).

Heat increment

The heat increment represents the energy expended during digestion, absorption, and metabolism of nutrients (van Milgen et al., 1997; Sakomura and Rostagno, 2017). The heat increment variations directly result from the feed composition, as the capacity of the feed to promote a thermal effect does not consider the maintenance fraction. Thus, as the heat increment does not represent an essential fraction for the bird, it is considered the system's inefficiency since this is a dietary energy that could be used for tissue deposition, also it is lost.

Energy systems

Poultry, such as broiler chickens, use energy from dietary nutrients through many mechanisms involved to process this energy. At each step of the energy flow on the animal, a fraction of this is expended and lost, which is used to characterize the feed energy expression systems.

From the study object's point of view (the animal), energy utilization results from the anabolic and catabolic pathways that provide sufficient nutrients (fuel) to cover the need for maintenance and growth. This fuel comes from nutrients that are substrates to be oxidized to produce energy in terms of ATP through exothermic processes (released heat) (van Milgen, 2002). Also, other fuel fractions can be lost before cellular oxidation, this on the excreta, which can be considered a potential energy source, but for many reasons, it is wasted.

As shown, diverse mechanisms are involved in energy utilization and influence the energy expression systems. It can be described in terms of the potential energy contained in the feed and effectively used by the animal to be stored as tissue and for maintenance. An adequate energy system is fundamental to formulating a balanced diet and optimizing feed efficiency (Guevara, 2004). In this sense, the energy value of feeds can be expressed in terms of gross energy (GE), apparent metabolizable energy (AME), and net energy (NE) (Sakomura and Rostagno, 2017; van der Klis and Jasman, 2019).

In this sense, it is important to understand the factors involved in energy metabolism: the feed value and the animal requirement. The energy system's accuracy and optimization depend on how closely the feed provides the energy and how close it is to the animal requirement (or utilization)(Figure 1).

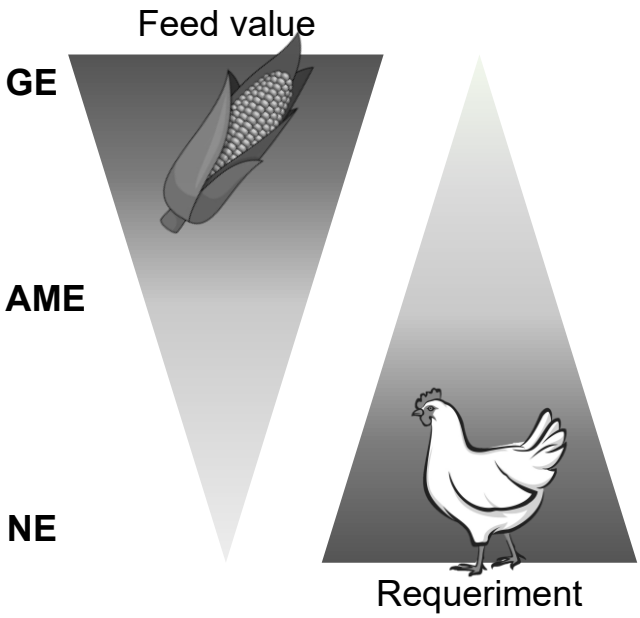

Figure 1. Schematic representation of the different energy systems used in poultry nutrition and the weighted interaction of the requirement of birds and the feed value (Adapted from van Milgen – Personal communication).

Gross energy (GE)

The GE of feed represents the total potential energy contained in a feed, as determined by complete combustion in an adiabatic calorimeter. That means the complete hydrolysis of the carbon-carbon linkage of the organic matter, principally components such as carbohydrates, fats, and proteins. This expression represents the maximum energy the diet provides due to the total oxidation. It is the first step to energy measuring on feeds and does not involve an animal factor in their determination.

GE is typically expressed in units of kilocalories (kcal) or megajoules (MJ) per kilogram (kg) of feed, as in the other system.

Metabolizavel energy

The metabolizable energy (ME) corresponds to the energy available to be used by the animal after subtracting the potential energy losses on the feces and urine, constituting

the excreta in birds. The determination of ME in feeds requires a biological assay, where the GE intake subtraction of the GE of the excreta is determined. In conceptual terms, this involves the energy that accounts for the digestibility and metabolic utilization of the nutrients.

Regarding methodological matter and conceptually, the ME can be expressed in apparent metabolizable energy (AME) and AME corrected by zero nitrogen retention (AMEn) (Anderson and Hills, 1980). The AMEn is the principal energy system used to express the energy value of feeds in poultry. D. C. Anderson and P. Hills proposed the AMEn in the 1960s, which accurately measured the energy evaluation of different feeds. This correction was explicitly attributed to poultry nutrition because birds, unlike most mammals, excrete nitrogen primarily in uric acid rather than urea, and the acid uric contains a significant amount of energy. The correction assumes that the AMEn provides a more accurate estimation of the feed energy value by adding back the energy equivalent of the nitrogen excreted in the urine. This adjustment is particularly significant in feeds with high protein content (Lopez, 2007).

Net energy

Net energy represents the energy available to the bird for specific basal physiological functions (maintenance) and the energy retained as tissues after accounting for the energy expended during digestion, metabolism, and physical activity (Sakomura and Rostagno, 2017) or minimum physical activity. Net energy values are determined by subtracting the energy lost in excreta, gases, and heat increment from the metabolizable energy of the feed. Net energy is considered the most accurate measure of available energy for birds that should be effectively used.

Effective energy

G. Emmans, a renowned researcher in animal nutrition, proposed the concept of an effective energy system to understand better and predict the energy utilization and requirements of animals, including poultry. The effective energy system considers the inefficiencies and variations in energy utilization and metabolic processes, providing a

more accurate representation of the "true" energy value available to the animal for productive purposes. This detailed description provides an overview of the effective energy system and its key components (Emmans, 1994). However, the effective energy system was developed and proposed for many species, starting from a theoretical utilization of nutrients and the fraction of fecal organic matter involved in energy determination. Their difficulties in interpretation limited their application in practical nutrition.

The practical implication of AME and NE

Traditionally, metabolizable energy has been used to measure dietary energy value in poultry diets. However, AME fails to account for the energy losses associated with the incomplete utilization of dietary nutrients and expended as heat increment. NE has emerged to address this limitation as a more accurate measure of dietary energy available for productive purposes in poultry.

NE provides a more precise estimation of feed ingredients and diets' "true" energy value or more optimized energy supply for best performance. NE systems have gained significant attention in recent years as they offer the potential for improved precision in formulating poultry diets and optimizing nutrient utilization (Carre et al., 2014; Wu et al., 2019; Noblet et al., 2023). Also, their practical implementation is already limited, and according to technological advances and research development, in the future, they will be used as they occur in swine nutrition. Additionally, the AMEn system works well under diets with ingredients that present a lower variation in their composition, like corn and soybean meal diets. One of the principal arguments is the challenge of the AMEn when is includes "non-traditional" ingredients in the feed formulation. Under these conditions, implementing NE can provide a feasible alternative for poultry nutrition.

Methodological tools to study NE in poultry

Several methods have been developed to determine the NE values of feed ingredients and diets for broiler chickens, starting with the principles of studies developed

for energy metabolism evaluations. These methods involve direct and indirect calorimetry techniques and mathematical models based on nutrient composition and digestibility data.

Calorimetry is the technique of measuring energy through direct and indirect ways. Direct calorimetry measures the heat production of chickens placed in calorimetric chambers, while indirect calorimetry estimates the heat produced indirectly based on oxygen consumption and carbon dioxide production (Guerrits et al., 2017). From the heat production determination and taking into account the other point of energy loss, it is possible to calculate the NE.

On the other hand, the comparative slaughter technique is a widely used method for determining NE values. The NE value of the tested diet is calculated by measuring the difference in energy retention during a specific period. From the retained energy, the heat production of each diet can be calculated, and consequently, the NE value can be calculated. This method provides valuable information on energy utilization efficiency and can be used to evaluate the impact of various feed ingredients on NE. Also, this method was questioned due to its many assumptions and results in limitations, being variability sources on the energy determination through methodological implications.

Indirect calorimetry (IC) has emerged as a valuable tool for studying poultry's energy metabolism and facilitating NE system development (Riveros et al., 2022; Riveros et al., 2023; Wu et al., 2019; Caldas et al., 2018). IC enables the direct measurement of gas exchange (O_2 consumption and CO_2 production) and the calculation of heat production based on the principle of stoichiometry. Accurately measuring heat production is crucial for estimating NE values, as it directly contributes to partitioning dietary energy. Moreover, IC allows for the assessment of energy utilization from feeds, evaluation of the effects of environmental conditions, and investigation of temporal variations in energy metabolism. Despite the progress made in NE systems and IC methodology, several challenges and limitations persist. Ensuring the quality and accuracy of IC results is essential for precisely estimating NE values and optimizing poultry nutrition.

Development of NE system for broiler chickens

In recent years, advancements in nutritional modeling and statistical analysis have led to the development of prediction equations for estimating NE values. These equations are based on the chemical composition of feed ingredients, such as crude protein, ether extract, crude fiber, and nitrogen-free extract, along with their digestibility coefficients. These prediction equations offer a practical and cost-effective approach to estimating NE values, particularly when conducting biological assays is not feasible.

Several studies have evaluated the NE values of different feedstuffs commonly used in broiler diets. For instance, Smith et al. (2019) investigated the NE values of corn, soybean meal, and wheat using indirect calorimetry and prediction equations. Their findings indicated that the NE values estimated by the prediction equations agreed with those obtained through indirect calorimetry. Similarly, Jones et al. (2020) evaluated the NE values of various oilseed meals, including canola, cottonseed, and sunflower meals. They demonstrated the influence of processing methods on the NE content.

Currently, laboratories across the world are engaged in developing an NE system for poultry. De Groot made a noteworthy contribution to this field in 1980, which later garnered interest in France, notably through the work of B. Carre in 2014. Carre compiled extensive data collected over the years, employing the comparative slaughter technique, and presented promising results that reignited enthusiasm among researchers. In Australia, significant advancements in developing NE equations for feeds were achieved at the University of New England by M. Choct, R. Swick, and S. Wu. Concurrently, research is underway in the USA at the University of Arkansas under C. Coon's laboratory, as well as in China and Thailand. In Brazil, efforts at São Paulo State University aim to establish a comprehensive NE system, yielding results that could potentially be applied to enhance poultry nutrition globally.

Conclusion

Accurate estimation of NE values is essential for formulating diets that meet the energy requirements of broiler chickens at different growth stages. Energy is a major

component of feed costs, and optimizing energy utilization can significantly improve feed efficiency and reduce production expenses. Furthermore, NE evaluations contribute to enhancing environmental sustainability by minimizing nutrient excretion and reducing the environmental impact associated with excessive feed energy supplementation.

In conclusion, net energy evaluation is crucial in formulating balanced and cost-effective diets for broiler chickens. Both direct calorimetry and prediction equations are valuable tools in estimating NE values of feedstuffs. Ongoing research and advancements in modeling techniques will continue to enhance our understanding of energy utilization in broiler chickens, ultimately leading to more precise and efficient feeding strategies.

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CHAPTER 2. Review: Energy Metabolism Evaluation Methods for poultry: from their principles to application

Review: Energy Metabolism Evaluation Methods for poultry - from their principles to application

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Abstract

The study of energy metabolism in animals, particularly poultry, has captivated researchers since the early 20th century. Numerous definitions and theories have emerged to quantify energy utilization in poultry birds. This review aims to compile and elucidate the fundamental concepts of energy metabolism in poultry, detailing the conceptual approaches and methodologies for *in vivo* evaluations across different bird categories and feeds. Through an exhaustive examination of existing literature and methodologies, this review aims to enhance our understanding and application of energy metabolism concepts in poultry research.

Keywords: Indirect calorimetry, heat production, energy expenditure, poultry metabolism

Introduction

Poultry nutrition is increasingly focused not only on improve bird performance but also to enhance sustainability by efficiently feed resources utilization. Energy, an important component on the feed, plays a critical role in improving feed utilization. Numerous studies have focused on determining the energy requirements of animals and feed energy values of feed (Sakomura, 2004; Noblet et al., 2022). Both components have been extensively evaluated in metabolizable energy (ME) bases.

Biological assays and protocols for determining feed energy value and bird requirements on an ME bases thought energy balance trials are well-described for different poultry categories (Hill and Anderson, 1958; Mateo et al., 2019). However, evaluating net energy (NE) requirements and feed value is still under development for poultry (Wu et al., 2019; Barzegar et al., 2019, Noblet et al., 2024; Cerrate et al., 2019; Riveros et al., 2023a). Historically, comparative slaughter technique was widely used to evaluate the energy utilization in birds (Sakomura et al., 2005; Reyes et al.,2011; van der Klein et al., 2020). Additionally, some indirect calorimetry (IC) method, initially developed for physiological studies (Farrel, 1972; Fuller et al., 1983), which has been successfully applied to poultry nutrition and metabolism studies (Caldas et al., 2018, Martinez et al., 2022, Riveros et al., 2023b). IC is a valuable tool that enhances data acquisition for studies determining the NE requirements of birds and evaluating the NE value of feeds. Both components should be evaluated complementarily thought standardized procedures to ensure accurate data collection, understanding their conceptual foundation, and being aware of their limitation and advantages.

This review delves into the conceptual foundations, principles, and tools essential for understanding and applying the methodologies used in studying energy metabolism in poultry. Specifically, we focus on the methodological tools of IC used in poultry trials, rather than on determining the energy value of feed or the models used to describe energy requirements and utilization, which have been well described in reviews by Sakomura (2004) and Zuidhof (2019), Noblet et al., (2022) and Barzegar et al., (2020). By examining these aspects, we aim to provide a comprehensive overview that can serve as material to be consulted by students, researchers, and technicians in the field.

Energy metabolism

Energy is an important component in animal feed, derived from macronutrients (carbohydrates, protein, and fats), essential for achieving desired production levels. Feed nutrients can either retain their structure for direct utilization or undergo structural modifications to synthesize other metabolites (McDonald et al., 2022). In the latter case, these nutrients can be partially or fully oxidized, releasing energy in the form of heat

(Chwalibog, 2002). Energy is fundamental for the basal functioning of an organism, contributing to growth, reproduction, and the production of substances such as milk, eggs, and other organic secretions (NRC, 1994).

Energy represents the potential power content in a fuel (nutrients) available to release heat or perform work. This energy enters metabolism and is regulated by various mechanisms, making it an intangible component with intricate dynamics. Understanding the nature of energy is crucial, as it is not a nutrient itself. Energy result from oxidation of fuel and the production of adenosine triphosphate (ATP), known as the "energy currency" of the cell (Seebacher, 2018). Cells use ATP as an energy transfer molecule through its hydrolysis and phosphorylation processes (Lehninger et al., 2008). ATP provides the necessary energy for various metabolic work task, supporting cellular multiplication, differentiation, and growth (Lehninger et al., 2008; Salin et al., 2015). While ATP represents the energy flow within the cell, its high dynamism makes it complex to measure-quantify, and represent the entirety of energy metabolism (Salin et al., 2015).

Therefore, the energy metabolism is quantified by measuring the energy intake by the animal and counting the energy outputs in excreta (feces and urine) and expended as heat, while and determining the partitioning of energy retained as body tissues. This approach provides a more comprehensible representation of the complex processes involved in the metabolic utilization of nutrients as energy.

Principles of energy metabolism

Multiple definitions elucidate the principles underlying energy metabolism, encompassing perspectives from chemistry, biochemistry, physiology, and thermodynamics. Regardless of the approach, energy metabolism can be characterized as an aerobic process occurring in the presence of oxygen, that converts nutrients from one form of energy to another. This conversion facilitates cellular work, synthesis of various components, and excretion of metabolic waste (Lehninger et al., 2008; Chwalibog, 2002).

To explain the oxidation more effectively, it is pertinent to revisit the insightful work of M. Kleiber (1961), who drew parallels between respiration and combustion. Kleiber observed that both the flame of combustion and animal respiration consume oxygen from the air while releasing water and CO_2 – a concept that remains valid today. Building on this, Brody (1945) experimentally demonstrated that the amount of heat produced per unit of O_2 consumed during combustion is nearly identical to that produced during animal respiration.

This understanding of energy metabolism is further supported by Hess's Law (1840), or the law of constant heat sums. This law postulates that the heat generated in a chemical process (be it respiration or combustion) is independent of the intermediate steps through which a system transits from its initial to final state. Both processes involve the complete breakdown of C-C bonds via complete combustion or complex enzymatic processes and pathways (McLean and Tobin, 1987).

Building on these foundations, it becomes feasible to quantify the caloric potential of principal macronutrients using adiabatic calorimeters based on the principle of total combustion. Additionally, this is the principal bases of IC method that is feasible to calculate the heat production (HP) from measuring gas exchange parameters (Gerrits and Labussière, 2015).

Units of energy measurement

Energy is broadly defined as the capacity to perform work (Atwater and Rosa, 1899). In this context, joules (J) are the SI unit of energy, defined as the energy transferred when a force one newton is applied over a distance of one meter (National Institute of Standards and Technology, 2019). Concurrently, another unit, calories (cal), is amount of energy required to raise the temperature of one gram of water by one degree Celsius at a pressure of one atmosphere (Atwater and Rosa, 1899). This measurement is particularly relevant to understanding energy transfer between systems, based on the temperature variations (McLean and Tobin, 1988).

In poultry science and industry, calories are typically used as the main unit to express energy requirements in kilocalories per day (kcal/day), and the energy content of the feed in kilocalories per kilogram (kcal/kg). The adoption of kcal can be attributed to the historical precedent set by the NRC (1994), which standardized this unit for expressing energy requirements. This has made kcal a familiar and widely accepted within the industry due to its simplicity and manageable numerical value. In contrast, using kilojoules (kJ) can result in larger, less convenient numbers (e.g., 3100 kcal/kg of feed equals 12958 kJ or 12.96 MJ) (Leeson and Summers, 2001).

Nutrients oxidation and stoichiometry

Oxidation refers to the breakdown of organic molecule that occur in the presence of O_2, producing CO_2, H_2O, and releasing heat through an exothermic process. In feed, nutrients (monosaccharides, fatty acids, and amino acids) undergo this process are typically represented by the macronutrients (carbohydrates, fats, and proteins) oxidation (see Figure 2). Carbohydrates after digestion and enzymatic hydrolysis in simple sugars, are primarily oxidized thought glycolysis, Krebs cycle and electron transport chain. Fats (triglycerides), after lipolysis, are oxidized through beta-oxidation in the mitochondria, transforming the carbon chain in glucose, which then enters the Krebs cycle. Protein is broken down into their monomers, oligomers, and amino acids. A significant proportion—up to half—of the ingested protein is metabolized in the liver through deamination to remove the amino group, and the remaining carbon chain enters the Krebs cycle for subsequent oxidation and energy production (Lehninger et al., 2008). This multi-pathway approach to nutrient oxidation highlights the complex metabolic pathways involved in energy production within the organism.

The equivalence between respiration and combustion makes possible to establish the stoichiometric balance by quantifying the amount (moles) of O_2 consumed and CO_2 produced from a specific macronutrient oxidation (Table 1). In practical terms, IC involves the volumetric measurement of gases exchanged (oxygen consumption – VO_2 and CO_2 production – VCO_2) during respiration, being feasible to calculate the energy expenditure of animals. This calculation is possible thought equation develop to estimate the HP from

VO_2 and VCO_2 (Table 2). One notable equation was proposed by Brower (1965), and is extensively used to calculate HP in humans and animals. This equation is based on older equation proposed by Weir (1949) with little variation on their constants. Additionally, other equations corrected for nitrogen excretion (Brower et al., 1965) and methane production (Lofgreen and Garret, 1968) were proposed but not widely implemented due to their complexity. Another variant equation proposed by Schmidt-Nielsen (1984) is used by comparative physiologists due to its development for different species. These tools are indispensable in research and practical applications, offering insights into the metabolic processes and energy requirements of various animal species.

Heat production from nutrients oxidation

The measurements O_2 consumption and CO_2 production during oxidative provide a robust foundation for HP calculation through IC method. This can be cross-referenced with the gross energy determined through total combustion analysis of nutrients (Gerrits and Labussière, 2015).

The caloric constants assigned to each macronutrient as shown in the Table 1, theatrically is correlate with the average HP from the total combustion of their respective monomers (Figure 1A). For instance, the monomers of carbohydrates generally exhibit minimal variation in their caloric output (3.73 to 4.18 kcal/g). The HP for carbohydrates such as starch, glycogen, lactose, fructose, and glucose is clustered around 4.0 kcal/g. This value aligns with the general caloric content of carbohydrates, which is approximately 4 kcal/g. Glycerol, which is not a typical carbohydrate but often included due to its role in lipid metabolism, also shows a similar HP. In contrast, fatty acids can display highest HP, ranging from 3.48 to 9.83 kcal/g. Shorter chain fatty acids (e.g., acetic acid - C2:0) have slightly lower HP compared to longer chain fatty acids (e.g., stearic acid - C18:0). This is consistent with the higher energy density of fats compared to carbohydrates and proteins. The HP for amino acids ranges from approximately 4.5 to 6.0 kcal/g. Amino acids like alanine (Ala), cysteine (Cys), and lysine (Lys) have HP near the lower end of this range. Amino acids such as phenylalanine (Phe) and tyrosine (Tyr) have higher HP, reflecting their more complex metabolic pathways.

This variation inside the same type of monomers is related with the number of carbons on their structure, e.g., saccharides presented five or six carbons (pentoses and hexoses), on the other hand, fatty acids can be presented from two to 20 carbons in their structure. The caloric constants for carbohydrates, fats, and proteins are 4.2, 4,4 and 9.5, respectively (Atwater and Byant, 1900), respectively. These values represent the average energy released during the complete combustion of the monomers that constitute these macronutrients.

However, the value of 5.6 kcal/g for protein is not commonly used in poultry nutrition. This constant is used in specific contexts, such as in the calculation of the caloric equivalent of nitrogen retention or in the context of body protein synthesis, rather than dietary energy content (Brower, 1965).

Respiratory quotient

The respiratory exchange ratio, or the respiratory quotient (RQ), is an important index of nutrients oxidation involved in metabolism. This ratio represents the volumetric or molar relationship between the CO_2 produced and the O_2 consumed. The RQ can be calculated from the stoichiometric balance of the nutrient oxidation, as depicted in Figure 1B.

For instance, during the oxidation of carbohydrates, six molecules of O_2 are needed to react with one glucose molecule, resulting in six molecules of CO_2. This stoichiometric balance results in an RQ typically close to 1 (Chwalibog et al., 2015). Commonly, RQ equal to 1 is associated for animals under feeding condition (Riveros et al., 2023a). Conversely, the RQ for fatty acids are generally lower, around 0.7 to 0.8, which is typical for fat metabolism. An RQ around 0.7 is associated with animals under fasting condition where body lipid is being catabolized. The variation among different fatty acids is relatively small compared to amino acids, suggesting more uniformity in their oxidation pathways. However, a significant proportion of animal fats is typically composed of palmitic acid (C16), stearic acid (C18), and monounsaturated oleic acid (C18:1) (Guessaman et al., 1988). Additionally, the average value of the RQ is slightly high due to the presence of

glycerol, which, although a minor constituent, is present in all fats (Gerrits and Labussière, 2015). The RQ for amino acids are more variable, ranging from 0.7 to 1.1. Amino acids like asparagine (Asn) and serine (Ser) have RQ close to 1.0, while others like methionine (Met) and lysine (Lys) have lower RQ values, indicating a greater O_2 consumption relative to CO_2 production. This variation reflects the diverse metabolic pathways of amino acids, including deamination and the urea cycle (Guessaman et al., 1988; Gerrits and Labussière, 2015). This variation is important to consider in metabolic studies, as it influences the overall RQ value and reflects the complex nature of animal metabolism and can interpreted on terms of type of substrate priority being oxidized and can indicate shifts in metabolic fuel utilization that is commented later (Figure 7).

Energy utilization

Figure 2 illustrates the pathways and components involved in energy metabolism in poultry, highlighting the digestion, absorption, and utilization of carbohydrates (CHO), proteins (CP), and fats (EE), along with their respective contributions to heat production (HP) and energy retention.

As shown, the digestible fraction (dCHO) that can be hydrolyzed into simple carbohydrates or monosaccharides (CHOsimple) is absorbed into the bloodstream. Carbohydrates are primarily metabolized to glucose, which enters glycolysis, producing pyruvate. Pyruvate is converted into Acetyl-CoA, entering the Krebs cycle and oxidative phosphorylation, producing ATP and heat (McDonald et al., 2022).

The digestible fraction of protein (dCP) represents the standardized ileal digestible essential amino acids (Essential AA SID) and digested non-essential amino acids (Dig non-essential AA). The essential AA SID can be metabolized to synthesize non-essential AA. Another fraction of the AA pool is available for deposition as body protein (PD) or expressed as ERpt. Amino acids can be used for protein synthesis or deaminated for energy. The carbon skeletons enter the Krebs cycle as various intermediates, producing ATP and heat, while nitrogen is excreted as uric acid.

Fats, expressed by the chemical determination of ether extract (EE), can be digested and broken down into fatty acids and glycerol. Fatty acids and monoglycerides are absorbed into the bloodstream. Fatty acids are converted into Acetyl-CoA through beta-oxidation, entering the Krebs cycle and producing ATP and heat. Glycerol can enter glycolysis. Another fraction of triglycerides is stored and deposited as lipid (LD) and retained as ERfat.

Acetyl-CoA (AcCoA), a crucial intermediary in the metabolism of carbohydrates, fats, and proteins, is central to integrating macronutrient metabolism, connecting carbohydrate, protein, and fat pathways to the Krebs cycle. From each nutrient pathway, HP is a byproduct of metabolic processes.

In general terms, the feed energy intake entails allocating a significant portion to increase body mass as ERpt and ERfat, maintaining essential metabolic functions and expending energy to develop their functions composing the HP (Rivera-Torres et al., 2010). To not enter in a complex macronutrient pathway, the energy partitioning refers to the detailed accounting of energy allocation as tissues or ER, the basal energy demand expressed by fasting heat production (FHP), as well as the heat increment (HI). The partitioning of energy into HP and ER (as protein and fat) facilitated to determining the energy efficiency of different feeds and the bird's requirement (McDonald et al., 2022).

Energy expression bases

The energy released from the complete breakdown of C-C bonds (complete combustion) is called gross energy (GE). GE of feed determination are conducted using an adiabatic calorimetric bomb.

When feed is ingested and some nutrients digested, discounted the fraction of energy lost in the feces is called digestible energy. In poultry, a portion of the digested nutrients not metabolically used is lost in the urine and expelled along with the feces, forming the excreta. For practical procedure reasons, the difference between GE intake and GE excreted is used to determine apparent ME. Finally, the fraction of ME discounting energy losses as HI is the NE that can be used for ER or for maintenance proposes (NEm)

(Latshaw and Moritz., 2009; Gerrits and Labussière, 2015). The partitioning of energy is illustrated in Figure 3.

Energy retention

A portion of energy is retained in the body as fat and protein tissue, increasing cell quantity (hyperplasia) and size (hypertrophy), contributing to an increase in the animal's mass. ER varies depending on the animal's growth rate and the duration of the evaluation period. There are notable variations in growing individuals, whereas variations tend to be more subtle in adult individuals (Tedeschi et al., 2019).

Heat production

The HP is a manifestation of the metabolic rate of an animal and is influenced by various factors, such as environmental temperature, feed characteristics, and the animal's physiological state (McLean and Tobin, 1988; Balnave et al., 1978). The HP is divided into three main components: FHP, thermal effect of feed or HI, and HPA, the processes involved in thermoregulation, and the energy expended due to physical activity (HPA), as illustrated in Figure 4 (Riveros et al., 2023a).

Partitioning of HP enables the study of the effects of various factors, which can be categorized as inherent to the animal (such as body mass, behavior, physiological state), dependent on feed characteristics (like physicochemical composition, particle size, and feed processing), and environmental factors (including temperature and photoperiod). Significantly, each of these factors predominantly influences a specific component of HP. Factors inherent to the animal typically induce several changes in the FHP, while feed factors mainly affect the HI. Additionally, animal behavior or physical activity directly impacts the HPA (Barrot et al., 1941; O'Neil et al., 1971; McLeod and Jewitt, 1974).

Fasting heat production

Fasting heat production represents the basal metabolic rate, the energy expended by birds to maintain vital physiological functions in a fasting state without the influence of physical activity (van Milgen et al., 1997; Balnave, 1978). This mechanism ensures a

constant flow of nutrient sources (expressed as energy) for essential physiological functions, including respiration, circulation, endocrine regulation, thermogenesis, neural function, and maintaining muscle tone (Cramton and Harris, 1969; Kil et al., 2013). FHP is determined under specific conditions where the animal should be at rest, in a post-absorptive state, not growing or reproducing, situated in a thermoneutral environment, and during an inactive phase of its circadian rhythm. The term NEm is commonly used in animal nutrition to denote the minimal energy requirements measured thought FHP, excluding any metabolic processes related to feeding and unaffected by diet characteristics (Johnson and Farrell, 1985; Noblet et al., 2015; Rivera-Torres et al., 2010). In this sense, NEm is expressed as a allometric function in terms of body weight (BW) denoted as $a*BW^b$ (Noblet et al., 2015). In Table 3, is summarized principal studies of FHP of different poultry species, highlighting the variability in the allometric scaling (b) and the NEm (a). Commonly for poultry, the allometric exponent of 0.75 is adopted to represent allometric scaling in adult animals, by their simplicity and applicable across different species. Also, slightly difference is reflecting in breed and poultry categories principally in growing and adult animals. In the same way, higher FHP is evidenced in fast growth rate of chickens than in adult birds.

Additionally, IC widely used for its accuracy and ability to measure real-time energy expenditure. It is particularly useful in controlled experimental settings (Noblet et al., 2015; Silva et al., 2024; Riveros et al., 2023; Rivera-Torres et al., 2010; Rivera-Torres et al., 2011). On the other hand, comparative slaughter and estimation of NEm can be useful for practical applications and broader studies but may introduce variability due to differences in methodology (Morris and Njutu, 1990; Sakomura et al., 2005).

Heat increment

The HI, also known as the thermal effect of feed, is described as the metabolic heat produced due to postprandial thermogenesis and the metabolic utilization of nutrients. The chemical composition of the feed primarily influences this effect. Van Milgen (1997) suggested partitioning the HI into the short-term thermal effect of feed, which is related to the ingestion and digestion process and results in the immediate release of heat following

feed ingestion. In contrast, the long-term thermal effect of feeding is defined as the absorption, mobilization, and utilization of nutrients that release heat during the post-absorptive phase and voluntary feeding pauses (but not under conditions of feed deprivation), as illustrated in Figure 4. Both components are related to the characteristics of the feed and represent a fraction of energy loss as heat (Labussiere et al., 2013; Gerrits and Labussiere, 2015).

The HI is an undesired fraction of energy in feed formulation, as it does not contribute to mass increase or production. This is considered an expression of the system's inefficiency, and various formulation strategies have been explored to reduce the HI. By managing the nutritional composition of the feed, it is possible to manipulate its HI (Figure 7). For example, feed with high EE result in lower HI, and previously mentioned, these diets present lower RQ associated with the oxidation of this sources (see stoichiometry). In contrast, high CP conte diets present high HI, with not marked influence on the RQ. Also, diets with high starch content induce in a higher RQ, and not marked variation on the HI.

Heat due to physical activity

The HPA contributes minimally to total HP, around 17% (Figure 4). However, its impact should not be disregarded. This is because physical activity can introduce variability, often referred to as 'noise', in the data collected during continuous measurements of HP. Additionally, the circadian rhythms regulated by the photoperiod where the animal express more active locomotion and behavioral expressions (see Figure 4) (McLeod and Jewitt, 1984). These observations underscore the importance of environmental and behavioral factors in assessing energy expenditure and overall animal welfare.

The evaluation of physical activity and the calculation of HPA help determine the "real" values of the fractions of HI and FHP without the effect of physical activity (st-FHP), which, as mentioned, can be a source of significant variation.

Methods for Energy Metabolism Evaluation

Understanding the fundamentals and components of energy metabolism is key to describing the methods used for its evaluation. However, it is crucial to acknowledge that each method has its limitations, which vary depending on the objectives of each study.

As previously mentioned, the chosen methods correspond to the measurement of different components of energy partitioning. For instance, the comparative slaughter technique is suitable for calculating energy retention. Conversely, indirect calorimetry is often the preferred method for real-time monitoring of variations in HP measurements. Ultimately, selecting a specific method depends on the availability of tools and equipment and the expertise of the researchers and technicians involved (Gerrits and Labussiere, 2015). This understanding ensures that the most appropriate and effective methodologies are employed to assess energy metabolism accurately in different research contexts.

Energy balance

The energy balance trial aims to measure the difference between the inputs (energy in feed) and outputs (energy in excreta), accounting them ER on their respective tissues (ERpt and ERfat). This method's primary objective is to assess input's effect on body composition (Tedeschi et al., 2019).

There are two main methods for computing ER. The first involves measuring the body composition in terms of protein and lipid content at both the beginning and end of the experiment and then multiplying these values by the caloric constant of each tissue type. The total energy retention is then calculated as the sum of the retained energy in each tissue type (Tedeschi et al., 2019). This method provides a comprehensive view of how dietary energy is utilized for tissue synthesis and maintenance in the body.

$$\mathrm{ER_{fat}} = (\mathrm{BL_f} - \mathrm{BL_i}) \times 9.1\ \mathrm{kcal/g}$$

$$\mathrm{ER_{pt}} = (\mathrm{BP_f} - \mathrm{BP_i}) \times 5.6\ \mathrm{kcal/g}$$

$$\mathrm{ER} = \mathrm{ER_{fat}} + \mathrm{ER_{pt}}$$

Where BL_i and BL_f are the total body content of fat at the beginning and final, respectively. BP_i and BP_f are the total body content of protein at the beginning and final, respectively.

The second way is to analyze the total gross energy of the body at the beginning ($GE_{body\text{-}i}$) and the final ($GE_{body\text{-}f}$) of the experiment and obtain the ER by the difference:

$$\mathrm{ER} = \mathrm{GE}_{\mathrm{body-f}} - \mathrm{GE}_{\mathrm{body-i}}$$

This method is practical and easy to implement, also present some limitations: (1) This process is labor-intensive, time-consuming, and ethically challenging due to the need for large numbers of animals (Emmans, 1995). (2) The method's accuracy relies heavily on precise measurements of body composition changes. Small errors in measuring the initial and final body compositions can lead to significant inaccuracies in estimating energy retention (Sakomura and Rostagno, 2017). (3) The requirement to sacrifice animals for body composition analysis raises ethical issues, particularly with increasing concerns about animal welfare in scientific research (Pomar et al., 2003). (4) Comparative slaughter is mainly applicable during specific growth phases and may not accurately reflect energy metabolism throughout the bird's entire lifecycle, especially in adult and non-growing birds (Noblet et al., 2015). (5) The method is subject to variability due to differences in individual bird metabolism, feed intake, and growth rates, which can affect the reliability of the results (MacLeod, 1997). (6) Comparative slaughter provides a static measure of energy retention and does not capture dynamic changes in metabolism over time, such as those occurring during different feeding regimes or stress conditions (Ferrell and Oltjen, 2008).

Indirect calorimetry

Indirect calorimetry is predicated on measuring respiratory gas exchange during a respiration trial, quantifying the VO_2 consumed, and VCO_2 produced by the animal. The characteristic of aerobic functions in animals enables the measurement of these gases with considerable accuracy. This method is advantageous, especially considering that O_2 and CO_2 gases can be measured more accurately than the small amounts of heat in direct calorimetry. Unlike energy or heat, these gases are not typically stored in the body

(McLean and Tobin, 1988; Lighton, 2008; Gerrits and Labussiere, 2015). IC present offers some advantages on their utilization as: (1) continuous monitoring of energy metabolism, capturing real-time changes in response to dietary and environmental factors (McLean & Tobin, 1987). (2) This method does not require sacrificing the birds, aligning better with ethical standards and allowing repeated measures on the same animals (Spratt et al., 1988). It provides comprehensive data on metabolic processes, including the thermic effect of feeding and physical activity, essential for a nuanced understanding of energy metabolism (Liu et al., 2017; Collin et al., 2003).

Several variants of indirect calorimeters have been developed, as depicted in Figure 5. Each variant operates on the principle of gas exchange measurements and offers advantages depending on the study's objectives. For instance, chamber variants such as negative (Figure 5A) and positive pressure (Figure 5B) can be used for group or individual measurements over medium to long evaluation periods (Riveros et al., 2023b). Conversely, masks provide more accurate measurements with minimal temporal delay but limit constant and voluntary feed intake (Figure 5C) (Nascimento et al., 2017). This restriction may impact the assessment of feeds and nutrients. Understanding these variations and their respective benefits and limitations is crucial for selecting the appropriate indirect calorimetry method for specific research needs.

Another type of respirometry chamber relates to how the air the animal will use is supplied inside the chamber. There are open and close-circuit systems of chambers of respirometry.

Open-circuit system

The open-circuit system is a setup where the primary sources of incoming gases (O_2 and CO_2) are atmospheric air. The concentration of this incoming air must be periodically monitored due to fluctuations in atmospheric composition, mainly due to variations in water vapor concentration, which are significant sources of variation due to atmospheric conditions. The incoming air is continuously recirculated, with the gas concentration and water vapor composition being recorded. The calculation of VO_2

consumption and VCO_2 production is based on the delta, which is the difference between the concentrations of incoming and outgoing gases expressed in terms of dry air (Figure 6A). Additionally, the advantage of this system lies in the fact that the airflow can be controlled at both incoming and outgoing points, based on the principle that the sum of both should be close to zero (Lighton 2008; Riveros et al., 2023b).

On the other hand, some advantages were mentioned, like real-time monitoring of metabolic parameters and energy expenditure, as soon as there is no need for specific gas concentration, which saves the cost of utilization.

Closed-circuit system

In closed-circuit systems, the animal is placed within a hermetically sealed chamber (Figure 6B). Air with a known concentration of a specific gas, typically from a certified oxygen source, is supplied. This setup can operate independently of analyzers, as the gas concentration is determined by the difference in volume or weight of any gas captured substance, from which the animal's VO_2 consumption and VCO_2 production are calculated. In this system, CO_2 is absorbed by a substance, often a combination of sodium hydroxide (NaOH) and potassium hydroxide (KOH). This substance "purifies" the air by removing CO_2 before recirculating it into the chamber (McLean and Tobin, 1988; Lighton, 2008; Gerrits and Labussiere, 2015). This design enables precise monitoring and control over respiratory gas exchange, rendering it an effective system for studying animal metabolism.

When using closed-circuit systems, a critical consideration is continuously monitoring the system's leaks. Even small leaks or the introduction of contaminants can significantly impact the accuracy of the VO_2 and VCO_2.

Additionally, there are notable advantages associated with the use of this system. It allows for real-time monitoring of metabolic parameters and is feasible for long-term measurements with high accuracy. These benefits make closed-circuit systems particularly valuable for detailed and extended studies of animal energy expenditure and metabolic processes.

Comparison between comparative slaughter and indirect calorimetry

On Figure 8 is represent different studies, demonstrating the variability and accuracy of these two methodologies in assessing energy metabolism in poultry, showing a strong correlation between RE measured by IC and RE calculated by MEI-HP (Barektaian et al., 2014; Liu et al., 2017). This indicates that indirect calorimetry can reliably estimate energy retention, often aligning closely with the results from comparative slaughter. However, slight discrepancies exist due to methodological differences. For instance, the MEI-HP approach might overestimate or underestimate energy retention depending on the precision of heat production measurements.

The variability in results, represented by different studies, highlights the influence of experimental conditions and bird species. For example, studies like Collin et al. (2003) and Spratt et al. (1988) show minimal deviation, suggesting consistent methodologies, whereas others like Caldas (2015) exhibit greater variability, possibly due to different calorimetry systems or experimental designs. The use of standardized protocols in indirect calorimetry ensures reproducibility and comparability across studies. This is crucial for establishing universal energy requirements and dietary recommendations (Noblet et al., 2015).

The choice between comparative slaughter and indirect calorimetry depends on the research objectives, ethical considerations, and resource availability. Indirect calorimetry, with its dynamic measurement capability and alignment with ethical standards, is increasingly preferred in poultry nutrition research. However, comparative slaughter continues to play a critical role in validating calorimetric models and providing foundational data on energy retention. Future research should focus on integrating both methods to leverage their strengths, ensuring comprehensive and accurate assessments of energy metabolism in poultry.

Conclusion

In conclusion, the comprehensive analysis of energy metabolism in animals, particularly through indirect calorimetry and the comparative slaughter technique, offers

valuable insights into how animals utilize dietary energy. While distinct in its approach, each method provides important information about energy metabolism.

Indirect calorimetry, advantageous for its real-time monitoring capabilities, measures HP and energy expenditure by assessing respiratory gas exchange. This method is particularly effective in determining the metabolic rate and the efficiency of nutrient utilization. However, its accuracy can be influenced by factors such as airtightness of the system and environmental variables. On the other hand, the comparative slaughter technique, which estimates ER by analyzing changes in body composition, is instrumental in understanding how nutrients contribute to tissue synthesis, particularly in the growth and development phases.

These methods, often used in conjunction, enable a holistic understanding of energy metabolism, facilitating effective nutritional strategies and improving animal welfare and productivity in various farming systems. The choice of method depends on the study's specific objectives, the available resources, and the required level of detail in the energy balance assessment.

Acknowledgements

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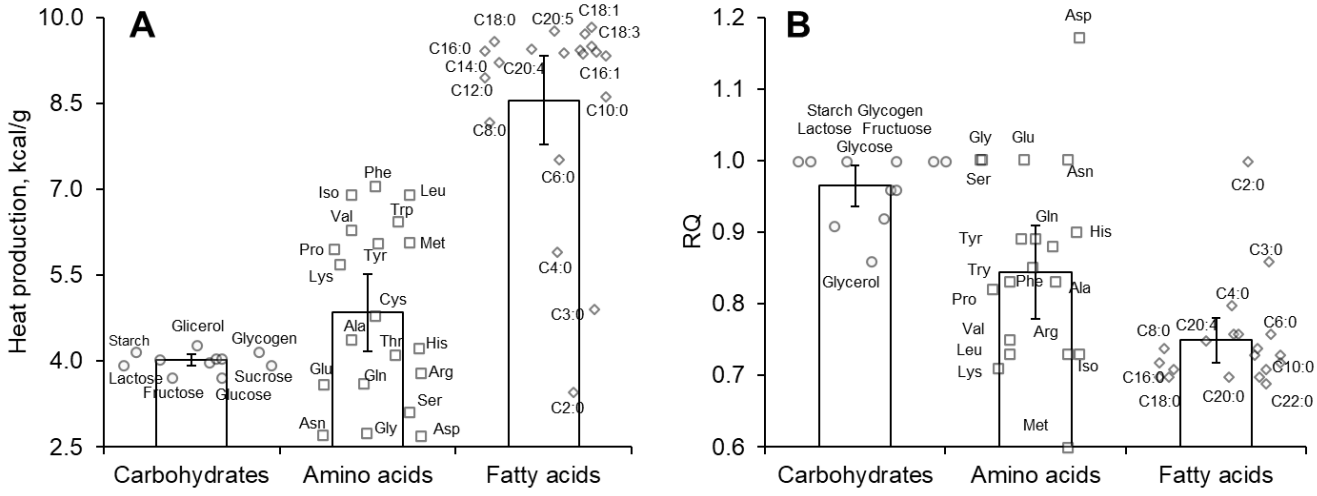

Figure 1. (A) Heat production and (B) respiratory quotient (RQ) from complete combustion of principal macronutrient monomers (amino acids, carbohydrates, and fatty acids). The confidence interval is 95%. Adapted from Gerrits et al., (2015), Blaxter (1989), Brody (1945) and Brouwer (1965).

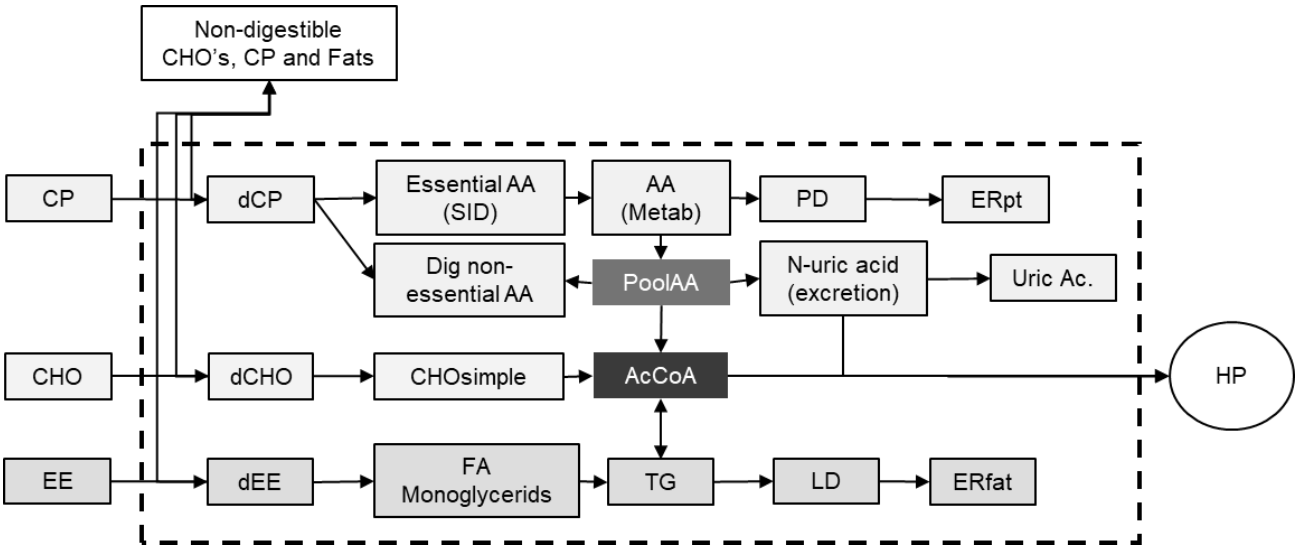

Figure 2. Flow of principal macronutrients (crude protein – CP, carbohydrates – CHO, and fats – represented by ether extract) and their relationship with energy metabolism.

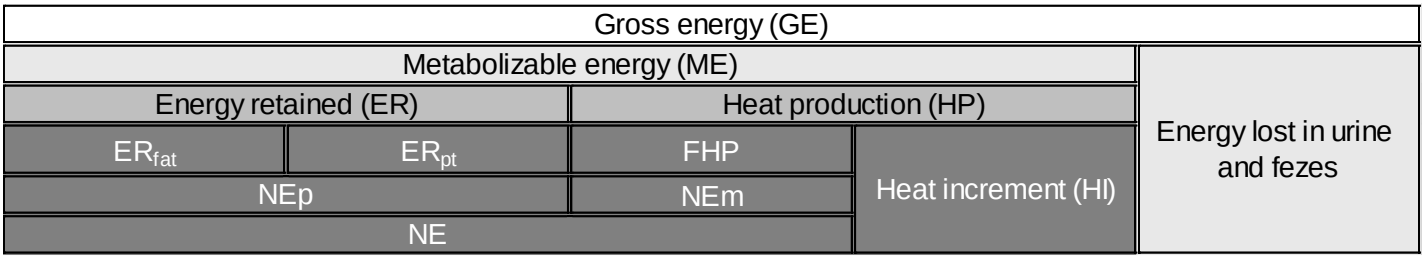

Figure 3. Feed energy partitioning. FHP: fasting heat production. NEp: net energy for production. NEm: net energy for maintenance. ERfat: energy retained as fat. ERpt: energy retained as protein. Eurine: energy from urine. Efeces: energy from feces.

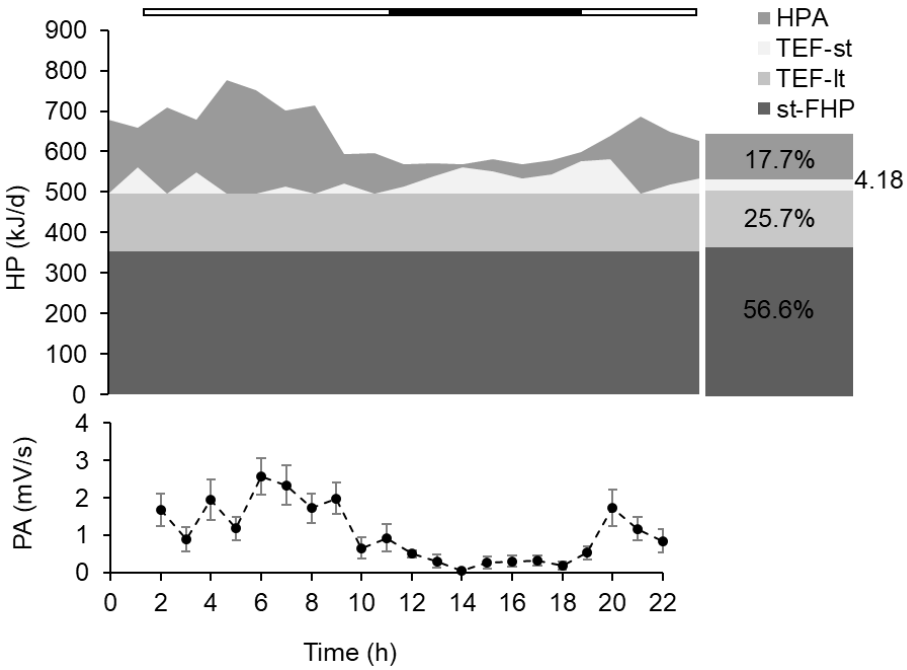

Figure 4. Heat production partitioning in meat-type roosters. st-FHP: fasting heat production standardized to zero physical activity. TEF-st: thermal effect of feed short-term. TEF-lt: thermal effect of feed long-term. HPA: heat due to physical activity. Adapted from Riveros et al., (2023a).

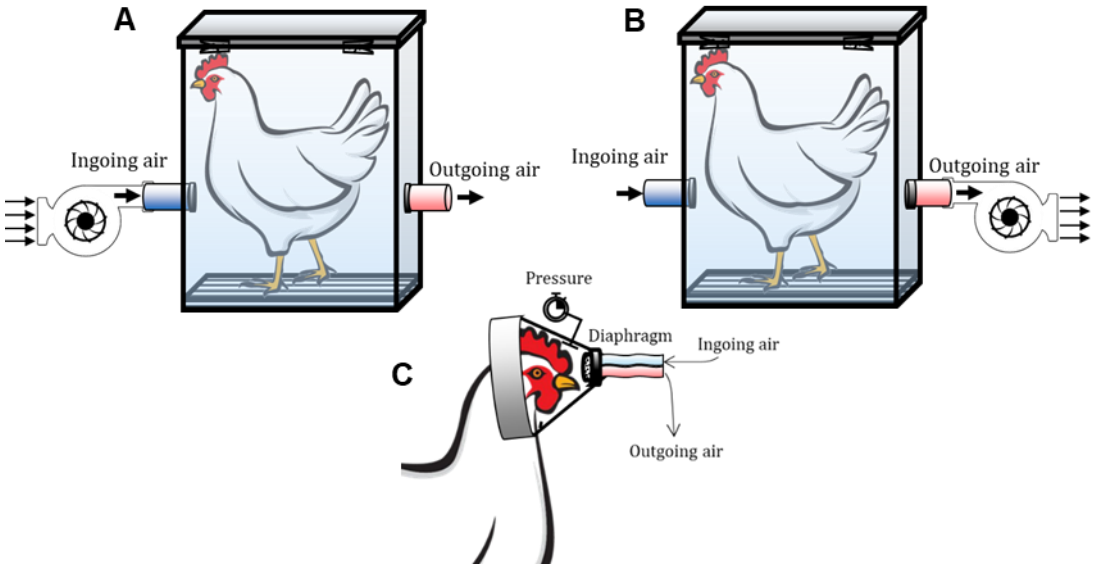

Figure 5. Scheme of typical glass chamber of respirometry pull mode (positive pressure) where the pump injects the air inside the camber (A) push mode (negative pressure) where the pump sucks the air from the chamber (B) and mask respirometer (C).

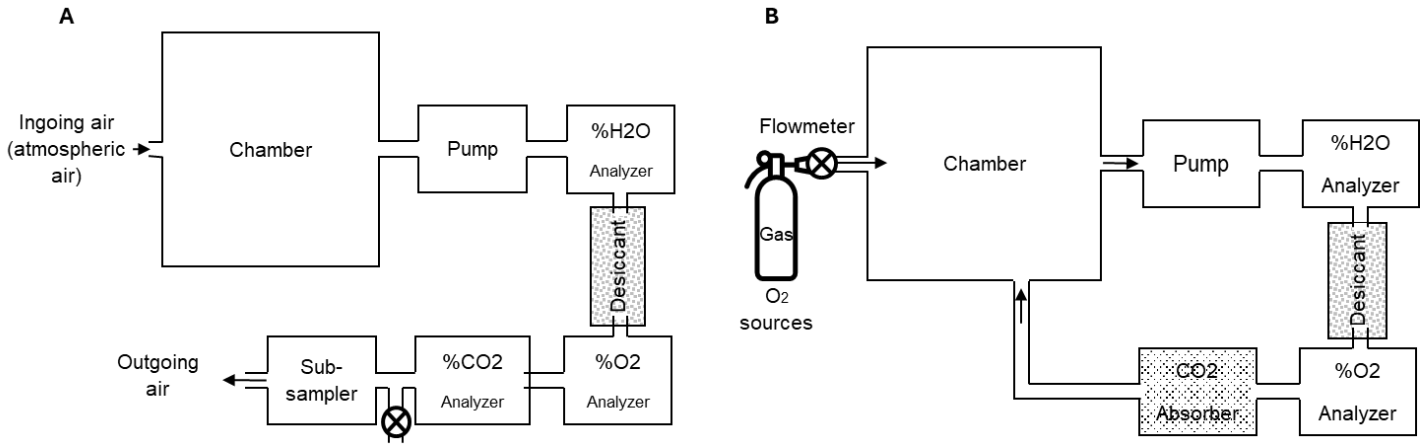

Figure 6. (A) Distribution and operation of an open-circuit indirect calorimetry system. The air usually passes to both O2 and CO2 analyzers. The system may include equipment to sample air from each chamber, pumping it to the analyzers. (B) Schematic distribution of a close-circuit indirect calorimetry system (subject to modifications). A gas (O2 sources) of known concentration is injected with a specific flow (and pressure) measured by a flowmeter inside the chamber and pushed by a pump and driver to analyzers. After gas passes through the CO2 analyzer, it is absorbed, and pure air is returned to the chambers.

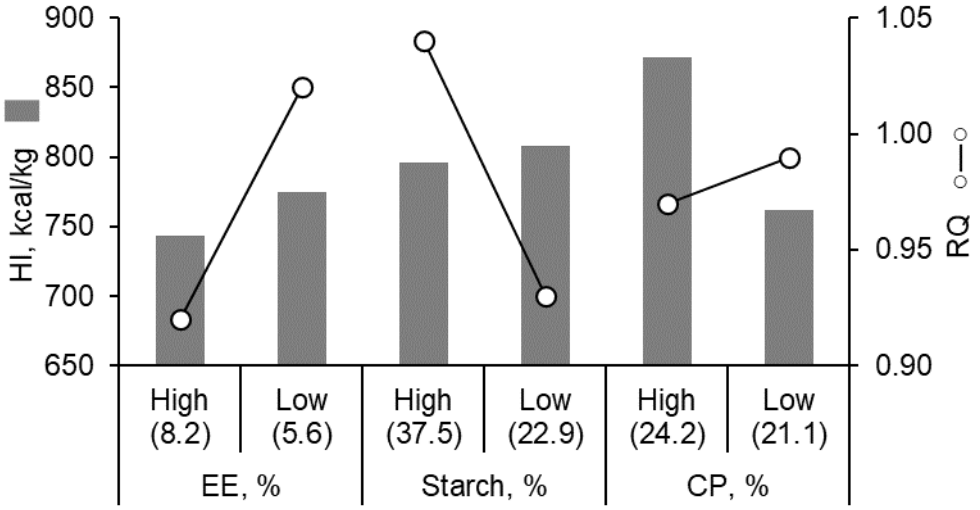

Figure 7. Effect of the nutritional composition of the feed on the heat increment and RQ. Adapted from Riveros et al., (Unpublished data).

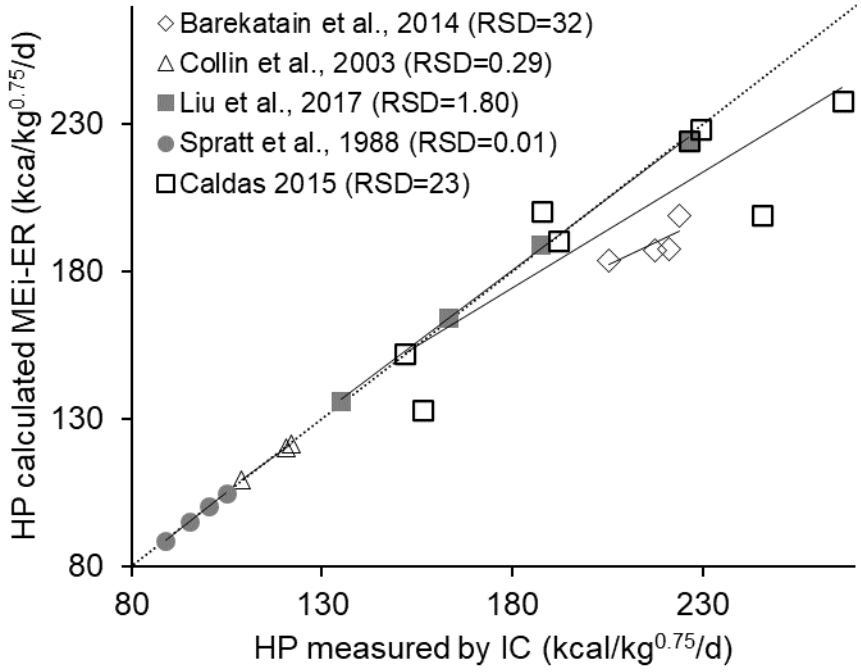

Figure 8. Comparison between energy retained (ER) measured by comparative slaughter (CS) and calculated by the difference of metabolizable energy intake (MEi) and heat production (HP) measured by indirect calorimetry. RSD: residual standard deviation.

Table 1. Stichometry of total oxidation of principal macronutrients and the heat released from the reaction. Negative signs represent exothermic reactions.

Nutrients (fuel)	Reaction	Heat (ΔH, kcal/g)
Carbohydrates	$C_x(H_2O)_x + 6O_2 \rightarrow 6CO_2 + 6H_2O + \Delta H$	-4.20
Protein	$R - CH(NH_2) - COOH + 0.8O_2 \rightarrow CO_2 + H_2O + NH_3 + \Delta H$	-4.40
Fat	$CH_3(CH_2)_xCOOH + 0.7O_2 \rightarrow CO_2 + H_2O + \Delta H$	-9.50

Table 2. Equations to calculate the heat production (HP, kcal) in function of gas exchange and other components develop in different species and can be used in poultry.

Sources	Parameters				Determined
	VO_2 (L)	VCO_2 (L)	N (g)	CH_4 (L)	
Weir (1949)	3.94	1.11			Human
Brouwer (1965)	3.87	1.20			Various livestock
Brouwer (1965)	3.87	1.20	-0.52		species
Lofgreen & Garrett (1968)	3.87	1.20	-0.52	2.45	Beef cattle
Schmidt-Nielsen (1984)	4.83	-1.23			Various species

$$\mathrm{HP\ (kcal)} = \mathrm{a} \times \mathrm{VO_2} + \mathrm{b} \times \mathrm{VCO_2} + \mathrm{c} \times \mathrm{N} + \mathrm{d} \times \mathrm{CH_4}$$

Table 3. Description of allometric exponent (a) and determination of fasting heat production (FHP) reported on the literature for poultry.

Bird Species	b	FHP (kcal/kg^b)	Sources	Method of determination
Domestic Chicken	0.75	70	Emmans, 1995	Theorical model estimation
Broiler Chickens	0.70	98-110	Noblet et al., 2015	Indirect calorimetry
Laying Hens	0.67	69	Morris and Njutu, 1990	Comparative slaughter
Meat-type growing chickens	0.60	117	van Milgen et al., 2001	Indirect calorimetry
Laying hens	0.75	85	Silva et al., 2024	Indirect calorimetry
Laying-type rooster	0.75	85	Riveros et al., 2023a	Indirect calorimetry
Broiler Chickens	0.75	90	Sakomura et al., 2005	Comparative slaughter
Growing turkey	0.66	110-113	Rivera-Torres et al., 2011	Indirect calorimetry
Growing turkey	0.77	106	Rivera-Torres et al., 2010	Indirect calorimetry

CHAPTER 3 - Technical Note: Description and validation of flow-through chambers of respirometry for measuring gas exchange in animal trials

Description and validation of flow-through chambers of respirometry for measuring gas exchange in animal trials

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Simple Summary

Indirect calorimetry method has been widely used for a long time in the study of energy metabolism in animals, and it remains an important tool for investigating energy metabolism and feed values. However, to ensure the quality of research data, it is necessary to standardize the calibration procedure. This paper presents a detailed procedure for calibrating and calculating indirect calorimetry data.

Abstract

Indirect calorimetry (IC) is a widely used method to study animal energy metabolism by measuring gas exchange. The accuracy of IC depends on detecting variations in signals reflecting the metabolic response, which can be challenging due to measurement noise and external factors. This study proposes a methodology to validate IC systems, including an easy-to-use spreadsheet for data computing, to verify accuracy and detect whole-system leaks. We conducted a recovery test using a simulation of CO_2 dynamics in MS Excel and injecting a known CO_2 concentration into four respirometry chambers. The thought flow rate of CO_2 was observed and compared to the expected rate from the simulation. Data filtering and computing, including a detailed calculation of signals calibration, Bartholomew transformation, and noise reduction, was developed to obtain the gas exchange and heat production parameters using an open-circuit IC system. The results from the recovery test in our system show that the proposed methodology is

accurate and precise. The proposed methodology and recovery test can be used to standardize the validation of IC systems together with adequate data computing, providing accurate measurements of animal energy metabolism in different environmental conditions and energy utilization from feeds.

Keywords: farm animals; gas exchange; energy expenditure; metabolic rate; respirometric chambers

Introduction

Indirect calorimetry (IC) is a widely utilized method for investigating the energy metabolism of animals and humans. It involves direct measurement of gas exchange (O_2 and CO_2) to calculate heat production (HP) based on the volumetric stoichiometry principle of oxygen consumed (VO_2), carbon dioxide produced (VCO_2), and heat released during the oxidative process [1]. Recently, IC has garnered interest among animal researchers as it supports studies on energy metabolism and the development of net energy systems [2-5].

IC enables accurate assessment of animal HP under different environmental conditions, energy utilization from feeds, and other temporal measurements [2,3,6]. Advancements in gas analyzer technologies, data acquisition systems, and computing power have enhanced measurement accuracy over the years [7,8]. However, ensuring the quality and accuracy of results necessitates the involvement of trained technicians to conduct biological trials, handle equipment appropriately, perform calibration procedures, and acquire data adequately [9].

The accuracy of an IC system depends on its ability to detect and record variations in signals reflecting the metabolic or physiological response of the animal, which the researcher interprets later [10,11]. Various procedures have been developed to assess IC accuracy, including alcohol or propane gas combustion, alcohol evaporation, continuous gas injection, and specific volume gas injection. However, some methods have limitations, such as low sensitivity, complex calculations, expensive materials or substances, and time-consuming procedures. Consequently, the recovery test should be flexible to

accommodate the diversity of IC systems and research objectives while simulating the experimental conditions of a biological trial, considering the flow used and the observed delta of CO_2 and O_2 during animal chamber measurements.

Inaccurate measurements or undetected system leaks within the IC setup can lead to misleading results, compromising the validity of animal energy metabolism studies. Erroneous measurements may result in overestimation or underestimation of energy utilization from feeds, potentially leading to suboptimal estimation of energy requirements. Therefore, the calibration procedure should align with the actual outgoing gas concentration the animal releases.

The open-circuit system integrated into the trough airflow of pull-mode calorimetric chambers is commonly employed in farm animal trials [2,3,6,9,12]. This system measures the concentration of gases (O_2 and CO_2) and their rate of change, considering the airflow from the atmosphere into the chamber. Subsequently, the volume of gas exchanged (VO_2 and VCO_2) over time can be calculated. However, before obtaining the final HP value, a series of computations are applied to preserve signals associated with the metabolic response, identify atypical signals resulting from extraneous factors, and suppress measurement noise [12-14].

This paper aims to establish a standardized methodology for validating IC systems, ensuring their accuracy, and detecting whole-system leaks. We propose using an easy-to-use spreadsheet for data acquisition and final HP calculation based on Brower's [1] fundamental equation. This approach yields an improved transient response and effectively suppresses measurement noise.

Materials and methods

General description of the flow-through IC system

We utilized an open-circuit indirect calorimetry (IC) system capable of connecting six chambers, as illustrated in Figure 1. Each chamber had an identical geometric volume (Vch) of 0.980 m^3 (dimensions: 100 cm×100 cm×98 cm). Inside each chamber, a

temperature control system comprising a heater and a cooler was implemented to maintain air temperature of 24±1.0°C and a relative humidity of 60% throughout the experimental trials.

The experimental setup consisted of air-conduction components, analyzers, and data acquisition equipment. Mass flow pumps (FK-100, Sable System, Las Vegas) were connected to each chamber and operated at a flow rate of 20 L/min, matching the measurements conducted on the animals. To ensure a consistent sample flow through the gas analyzers, an air sample of 160±2.0 mL/min was extracted from each flow pump using a sub-sampler pump (SS4, Sable System, Las Vegas) positioned at the end of the circuit. The extracted air sample underwent analysis of water vapor pressure using an RH-100 device (Sable System, Las Vegas). The humid air was subsequently passed through a drying column filled with >99.5% $CaSO_4$ (Drierite®) to remove humidity and enable an analysis of dry air concentration.

The concentrations of O_2 and CO_2 were analyzed from the dry air sample using paramagnetic (PA-10, Sable System, Las Vegas) and infrared (CA-10, Sable System, Las Vegas) analyzers, respectively. A universal interface (UI-3, Sable System, Las Vegas) was connected to the flowmeters and analyzers to record the signals at a frequency of one record per second. The signals from the analyzers and flowmeters were extracted using ExpData software (Sable System, Las Vegas).

The atmospheric air was conducted using a diaphragm pump, and O2 and CO2 concentrations were measured to establish the baseline concentrations.

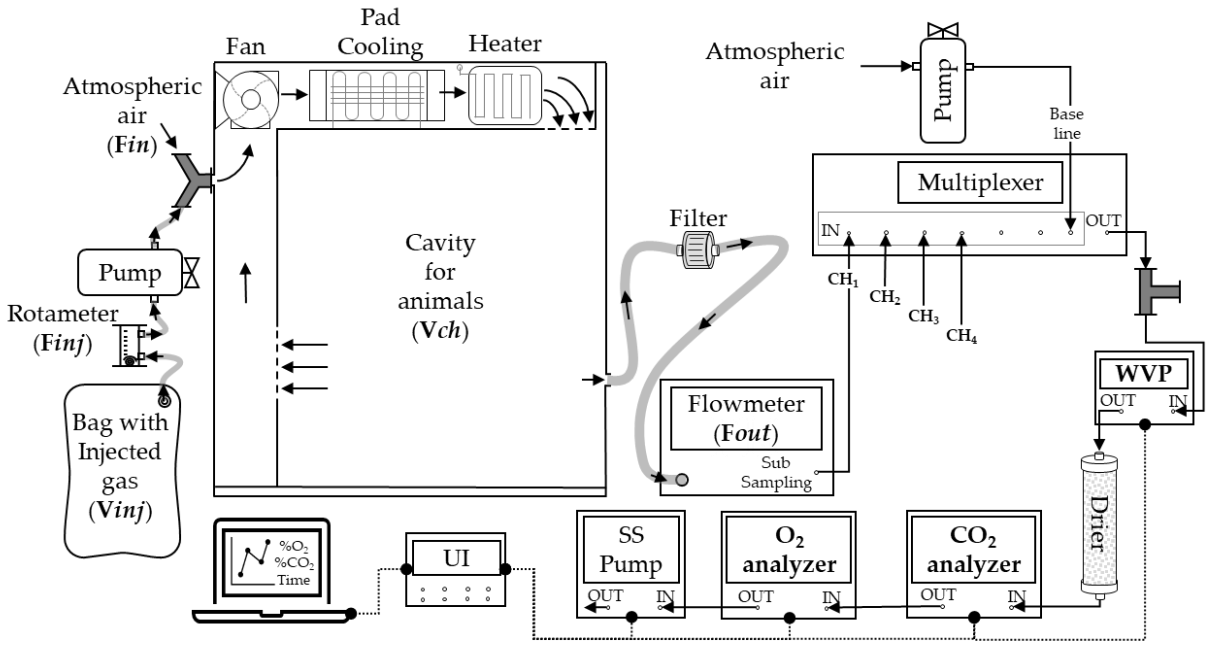

Figure 1. Scheme of multiple flow-through respirometry systems and coupling to the gas for injection test. Fin: ingoing flow. Fout: outgoing flow. Finj: injection flow. CH_i: chambers (i = 1, 2, 3, 4). WVP: water vapor pressure analyzer. The arrows represent the airflow direction (→). Data transference line (●---●).

General calculations

The calculations employed in this study for simulating gas injection dynamics and data computation were based on the methods described by Ligthon [15] for an open-circuit system operating under negative pressure.

The correction of the outgoing flow (Fout) by barometric (BP) and water vapor pressure (WVP) was calculated as $\mathrm{Fout} = \mathrm{Fout}_{(\mathrm{dyl})} * \frac{\mathrm{BP}}{\mathrm{BP}-\mathrm{WVP}}$; where $\mathrm{Fout}_{(\mathrm{dyl})}$ is the outgoing flow of wet air. The ingoing flow (Fin) was determined by the nitrogen correction factor using the equation $\mathrm{Fin} = \mathrm{Fout} * \left(\frac{\mathrm{N_2 in}}{\mathrm{N_2 out}}\right)$, where $\mathrm{N_2} = 100 - \mathrm{O_2} - \mathrm{CO_2}$, disregarding the minor components of atmospheric air (e.g., Ag, CO, H_2, CH_4, etc.).

The volume of gases (L/min) was determined by multiplying the airflow and their respective gas concentration: $\mathrm{VCO_2 out} = \mathrm{Fout} \times \mathrm{CO_2 out}$, $\mathrm{VCO_2 in} = \mathrm{Fin} \times \mathrm{CO_2 in}$, and

similarly for oxygen. The oxygen consumption (VO_2) was calculated from the volumetric difference between ingoing and outgoing gases: $\mathrm{VO_2} = \mathrm{Fin} \times \mathrm{O_2in} - \mathrm{Fout} \times \mathrm{O_2out}$, and CO_2 production (VCO_2) was determined as $\mathrm{VCO_2} = \mathrm{Fout} \times \mathrm{CO_2out} - \mathrm{Fin} \times \mathrm{CO_2in}$.

During the recovery test, the volume of injected CO_2 (VCO_2inj) was computed as $\mathrm{VCO_2inj} = \mathrm{Finj} \times \mathrm{CO_2inj}$; where Finj is the controlled injection flow, and CO_2inj is the known concentration of tested gas.

The HP was calculated based on the volumes of gas exchange using Brower's fundamental equation [1]: $\mathrm{HP(kcal)} = 3.866 \times \mathrm{VO_2} + 1.200 \times \mathrm{VCO_2}$.

Simulation of the dynamic of gas injection in a theoretical system

To perform the recovery test, a simulation was developed using Microsoft Excel spreadsheet (S1 File). The simulation was based on a theoretical system assuming constant flow with no significant resistances or leaks ($\mathrm{Fin} = \mathrm{Fout}$ or $\frac{\mathrm{N_2in}}{\mathrm{N_2out}} = 1$), and dry air passing through the system ($\mathrm{WVP} = 0$). This allowed us to describe the behavior of the injected CO_2 concentration (CO2inj) over time (per minute). Parameters such as the volume of injected gas (Vinj), Finj, and CO_2inj concentration were kept constant but can be modified for testing other gases based on the study's objectives, system characteristics, and simulated scenarios. Additional calculations are detailed in Table 1.

The simulation was conducted to determine the volume of CO_2 in three compartments: (1) ingoing volume (VCO_2in), (2) CO_2 volume in the chamber (VCO_2ch), and (3) outgoing volume (VCO_2out) (Figure 2, representation A). The simulation consisted of two phases: the injection phase (when Finj > 0 and ti < tinj) and the washing phase (when Vinj was empty at ti > tinj and Finj = 0) (Figure 2, representation B).

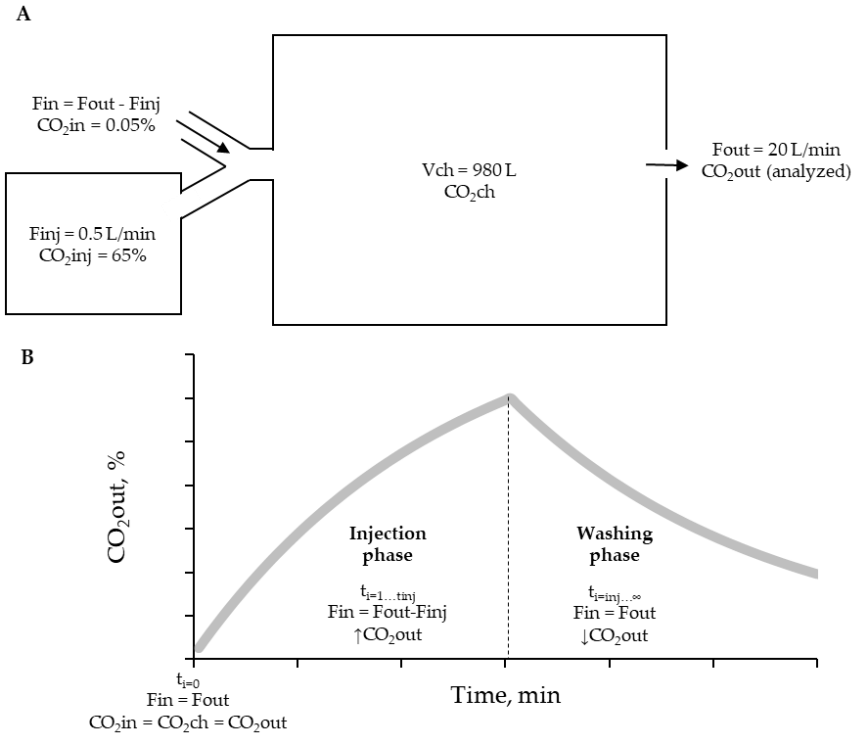

Figure 2. **A.** Illustrative scheme of the recovery procedure with an injection of a known gas concentration (65% CO_2). Fin: ingoing flow. Fout: outgoing flow. Finj: pure gas injection flow. V_{CH}: chamber volume. Vbag: volume of the bag that contains tested gas. → airflow direction. **B**. Phases of CO_2 recovery test and CO_2out behavior defined by the simulation. The tinj differentiates the injection and washing phases.

At the start of the simulation (ti = 0), representing the absence of gas injection or baseline condition, the concentration of CO_2 in all compartments was equal to the atmospheric air concentration. The volumetric content of each compartment was established as follows:

$$\mathrm{VCO_2}_{\mathrm{ti=0}} = \begin{cases} \mathrm{VCO_2in\ (L/min)} = \mathrm{Fout} \times \mathrm{CO_2in}\ ;\ \mathrm{Finj} = 0;\ \mathrm{Fout} = \mathrm{Fin} \\ \mathrm{VCO_2ch\ (L)} = \mathrm{Vch} \times \mathrm{CO_2in} \\ \mathrm{VCO_2out\ (L/min)} = \mathrm{Fout} \times \mathrm{CO_2out};\ \mathrm{CO_2in} = \mathrm{CO_2out} \end{cases}$$

For the injection phases (ti = 1 to tinj), the following calculations were performed:

$$\mathrm{VCO_2}_{\mathrm{ti=1\ldots tinj}}$$

$$= \begin{cases} \mathrm{VCO_2in\ (L/min)} = (\mathrm{Fout} - \mathrm{Finj}) \times \mathrm{CO_2in} \\ \mathrm{VCO_2ch\ (L)} = \mathrm{VCO_2ch_{ti-1}} + \mathrm{VCO_2in_{ti}} + \mathrm{VCO_2inj_{ti}} - \mathrm{VCO_2out_{ti-1}};\ \mathrm{VCO_2inj_{ti}} = \mathrm{Finj} \times \mathrm{CO_2inj} \\ \mathrm{VCO_2out\ (L/min)} = \mathrm{Fout} \times \mathrm{CO_2ch_{ti}};\ \mathrm{CO_2ch} = \dfrac{\mathrm{VCO_2ch_{ti}}}{\mathrm{Vch}} \end{cases}$$

In the above equations, VCO_2ch at t=1 was calculated by summing the volume of CO_2 in the chamber (VCO_2ch) at t=i-1, the VCO_2in at t=i, and the VCO_2inj at t=i, and then subtracting the VCO_2out at t=i-1. The VCO_2out at t=i was used to determine the fractional concentration of CO2 in the chamber (CO_2ch) at t=i, calculated as $\mathrm{CO_2ch} = \frac{\mathrm{VCO_2ch}}{\mathrm{Vch}}$.

During the washing phase (ti = tinj+1 to infinity), the CO_2 volumes were calculated as follows:

$$\mathrm{VCO_2}_{\mathrm{ti=tinj+1}\to\infty} = \begin{cases} \mathrm{VCO_2in}\ (\frac{\mathrm{L}}{\mathrm{min}}) = \mathrm{Fin} \times \mathrm{CO_2in} \\ \mathrm{VCO_2ch\ (L)} = \mathrm{VCO_2ch_{ti-1}} + \mathrm{VCO_2in_{ti}} - \mathrm{VCO_2out_{ti-1}} \\ \mathrm{VCO_2out}\left(\dfrac{\mathrm{L}}{\mathrm{min}}\right) = \mathrm{Fout} \times \mathrm{CO_2ch_{ti}};\ \mathrm{CO_2ch} = \dfrac{\mathrm{VCO_2ch_{ti}}}{\mathrm{Vch}} \end{cases}$$

The outputs of interest from the simulation over time included CO_2out, the differential volume of CO_2 ($\Delta\mathrm{VCO_2}_{\mathrm{ti}} = \mathrm{VCO_2out_{ti}} - \mathrm{VCO_2in_{ti}}$), and the cumulative differential volume of CO_2 ($\mathrm{Cumulative}\Delta\mathrm{VCO_2} = \sum_{\mathrm{ti=0}}^{\mathrm{i}} \Delta\mathrm{VCO_2}_{\mathrm{ti}}$). These parameters, calculated per minute, were used for comparing the result of each chamber.

Table 1. Parameters and calculations used for the simulation of recovery test over time (ti).

Variable	Description	Parameter or calculation	Units
Initial parameters for the simulation			
Atmospheric CO_2 or fractional ingoing concentration	CO_2in	0.05	%
Atmospheric oxygen or fractional ingoing concentration	O_2in	21	%
Outgoing airflow (dry air)	$Fout_{(ti)}$	20	L/min
Volume of injection	Vinj	30	L
Fractional concentration of CO_2 injected	CO_2inj	65	%
Injection flow for ti < tinj	$Finj_{(ti)}$	0.5	L/min
Intermediate calculations for t=i			
Injection time	tinj	Vinj/Finj = 60	min
Ingoing volume of CO_2	$VCO_2in_{(ti)}$	$Fin_{(ti)}*CO_2in_{ti}$	L/min
Injected volume of CO_2	$VCO_2inj_{(ti)}$	$Finj_{(ti)}*CO_2inj_{ti}$	L/min
Volume of CO_2 in the chamber	$VCO_2ch_{(ti)}$	$VCO_2ch_{ti-1}+VCO_2in_{ti}+VCO_2inj_{ti}-VCO_2out_{ti-1}$	L
Fractional concentration of CO_2 in the chamber	$CO_2ch_{(ti)}$	VCO_2ch/Vch	%
Outgoing volume of CO_2	$VCO_2out_{(ti)}$	$Fout_{ti}*CO_2ch_{ti}$	L/min
Outputs			
Fractional concentration of outgoing CO_2	$CO_2out_{(ti)}$	CO_2ch_{ti-1}	%
Differential volume of CO_2	ΔVCO_2	$VCO_2out_{ti} - VCO_2in_{ti}$	L/min
Cumulative volume of differential CO_2	Cumulative ΔVCO_2	$\Sigma\Delta VCO_2ti\rightarrow\infty$	L

Recovery test procedure

The recovery test was conducted in four respirometry chambers to assess the accuracy and precision of the system by injecting a known concentration of CO_2 and measuring the rate of gas ingoing and outgoing over time. The recovery protocol was designed with the following considerations: (i) continuous monitoring and control of the gas concentration and injection flow rate at every time unit (each second), (ii) ensuring that the concentration and injection flow rate fall within the expected range of metabolic rates observed in animal trials, and (iii) comparing the simulated flow rate of CO_2 with the observed rate in each chamber to evaluate the accuracy and precision of the system.

A non-diffusion medical bag (Jiangsu Yuyue Medical Equipment & Supply CO., LTD., Nanjing, China) was used with a capacity to store 30 L of a known CO_2 concentration (65% analytical CO_2 and 35% compressed nitrogen, standard gas mixture

with guaranteed concentration, Code: ONU-1013, White Martins, SP, Brazil). The bag was connected to a micro-diaphragm pump with a pressure of 90 kPa (CTS Parker Hannifin, Ohio, USA). It was used to inject the gas into the chamber at a controlled injection flow rate of Finj=0.5 L/min, which was monitored using a rotameter (Figures 1 and Figure 2, representation A). After that, the data collection was started by 60 minutes (injection time) up to the bag empty and continued for another 60 minutes without injection (washing time). This process was repeated three times in each chamber, and the average of the three observations was compared with the expected behavior of CO_2out, ΔCO_2out, and Cumulative ΔCO_2 obtained from the simulation at each time point (*t*i).

Data analyses and recovery index calculation

The data analysis for the recovery test followed the same calculation procedures as described in the simulation. Data was recorded at one-second intervals and then averaged every minute. Several parameters were evaluated to assess the accuracy of the system in each chamber, including the fractional concentration of CO_2out, ΔVCO_2, and cumulative ΔVCO_2. The error (ε) and residual standard deviation (RSD) were calculated for each chamber and minute to evaluate the results.

The error (ε) was calculated as the difference between the observed and expected (simulated) value:

$$\varepsilon(\mathrm{k}) = \mathrm{k}_{\mathrm{observed}} - \mathrm{k}_{\mathrm{expected}}$$

The residual standard deviation (RSD) was determined by taking the square root of the sum of squared differences between the observed and expected values, divided by the sample size (n), as follows:

$$\mathrm{RSD}(\mathrm{k}) = \sqrt{\frac{\sum\left(\mathrm{k}_{\mathrm{observed}} - \mathrm{k}_{\mathrm{expected}}\right)^2}{\mathrm{n}}}$$

In each chamber, both ε and RSD were calculated for CO_2out(*ti*), ΔVCO_2, and Cumulative ΔVCO_2.

Additionally, the recovery rate was determined by comparing the observed volume of CO_2 (ΔVCO_2 observed) with the expected volume of CO_2 (ΔVCO_2 expected) in each chamber. The recovery rate was calculated over a period of 120 minutes using the following formula:

$$\text{Recovery rate} = \frac{\sum_{i=0}^{120} \Delta VCO_2 \text{observed}}{\sum_{i=0}^{120} \Delta VCO_2 \text{expected}}$$

Here, k represents CO_2out(*ti*), ΔVCO_2, or Cumulative ΔVCO_2. The observed values were recorded in each chamber, while the expected values were obtained through the simulation.

The procedure of data computing of a multi-chamber IC system

For this procedure, we used the IC data report presented by Camargos et al. [17] on broiler chickens to illustrate the step-by-step calculations involved in data management. The calculation spreadsheet developed in MS Excel containing this data is available as supplementary material (S2 File).

The signals extraction of the fractional concentration of O_2 and CO_2, Fout (of each chamber), BPA, and WVP, exported one data per second to MS Excel. Since the experimental setup involved a multi-chamber IC system, certain inputs were necessary to define the recording sequence of the analyzers, which was controlled by a multiplexer, and to enable automated data processing. These inputs were incorporated into the MS Excel spreadsheet. Specifically, the programmed time sequence in ExpeData was provided within the MS Excel spreadsheet. This sequence dictated the recording of gas concentrations during both the baseline and chamber measurement periods. In the example provided in S2 File, the program was set to include an initial and final baseline (each with a reading duration of 180 seconds) and to record the gas concentrations in each chamber between the baselines (each with a reading duration of 540 seconds).

Consequently, a complete cycle of gas concentration readings for all chambers lasted for 60 minutes (Figure 3), and this cycle was looped continuously for 24 hours.

Detailed information regarding the chamber codification, sequence of inputs, and additional parameters (e.g., chamber volume and the body weight of animals inside the chambers) can be found in the S2 File.

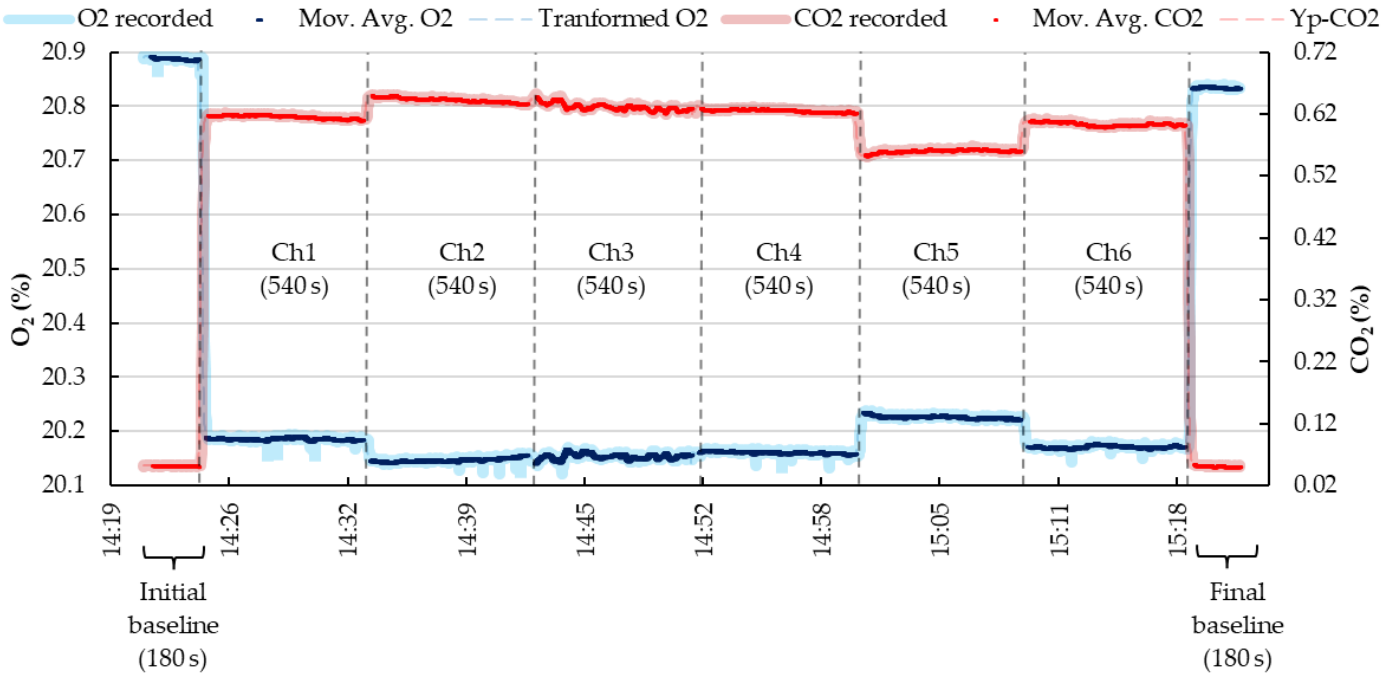

Figure 3. Example of recording, transforming, and filtering the gas concentration sequence in a multi-chamber IC system. The black dashed line represents the recording time limit between chambers and the baseline.

Oxygen and CO_2 signal calibration

The initial step in conducting IC measurements involved verifying the recorded signals and ensuring consistent gas concentration values. To achieve this, the analyzers were calibrated at the beginning and end of each metabolic measurement period. In the study conducted by Camargos et al. [17], calibration procedure was performed daily.

The calibration procedure employed two gases with known certified concentrations: Gas A, which consisted of pure nitrogen with approximately 99.99% N_2, 0% O_2, and 0% CO_2 (White Martins, SP, Brazil), and Gas B, a standard mixture comprising 21% O_2 and 1% CO_2 (White Martins, SP, Brazil).

For each gas of interest (O_2 and CO_2), a calibration curve was generated by extrapolating the concentrations over time (*t*i) using the following equation:

$$\mathrm{CO_2}\ \text{extrapolated for}\ t\mathrm{i}\ (\text{for gas A or B}) = \mathrm{CO_2}t0 + (\mathrm{CO_2}t\mathrm{n} - \mathrm{CO_2}t0) \times \frac{t\mathrm{i} - t0}{t\mathrm{n} - t0}$$

Here, CO_2*t*i represents the fractional concentration of Gas A or Gas B at time *t*i. At the same time, CO_2*t*0 and CO_2*t*n denote the concentrations recorded by the analyzer at the initial and final time points for Gas A and B, respectively. The extrapolated CO_2 concentration for each ti and gas (A and B) was then linearized as follows:

$$\text{slope}\ (t\mathrm{i}) = \frac{(\mathrm{CO_2}\text{concentration for gas A} - \mathrm{CO_2}\text{concentration for gas B})}{(\mathrm{CO_2}\text{extrapolated at}\ t\mathrm{i}\ \text{for gas A} - \mathrm{CO_2}\text{extrapolated at}\ t\mathrm{i}\ \text{for gas B})}$$

$$\text{intercept}\ (t\mathrm{i}) = \mathrm{CO_2}\text{concentration for gas A} - \text{slope}\ (t\mathrm{i}) \times \mathrm{CO_2}\text{extrapolated at}\ t\mathrm{i}\ \text{for gas A}$$

Finally, the calibrated signals and expression of the gas concentrations were represented as:

$$\mathrm{CO_2}\text{calibrated}\ (t\mathrm{i}) = \text{intercept}(t\mathrm{i}) + \text{slope} \times \mathrm{CO_2}\text{register}\ (t\mathrm{i})$$

The same procedure was applied to calibrate the O_2 signals. In O_2 and CO_2 measurements, the fractional concentrations derived from the calibration process were used for subsequent calculations.

Calibrated fractional concentration and filtering

The fractional concentration obtained through calibration alone is insufficient for calculating heat production (HP). Therefore, the reliability of the IC measurements relies on the detection of metabolic signals by the system or the appropriate mathematical techniques applied to highlight these metabolic events.

The Bartholomew transformation [18] is a commonly used procedure for real-time gas exchange measurements [19]. This transformation is based on the relationship between Vch and Fout, representing the system's ability to detect the metabolic signal or provide a delay for corrective action in its absence.

The concentrations of O_2 or CO_2 can be corrected by incorporating the exponential saturation of the chamber, which is dependent on Fout. The calibrated concentrations of O_2 and CO_2 at a specific time (*t*i) can be corrected using the following equation:

$$\mathrm{CO_2}(ti)\ \mathrm{transformed} = \frac{\mathrm{CO_2calibrated}\ (ti) - \mathrm{CO_2calibrated}\ (ti-1)}{1 - \mathrm{e}^{-\frac{\mathrm{Fout}(ti)}{\mathrm{Vch}} \times ti}}$$

After applying the Bartholomew transformation, the CO_2 concentration is transformed to the time *t*i. Since the signals were recorded every second, a moving average (n=10) was employed as a criterion to reduce noise and synchronize the O_2 and CO_2 analyzer signals.

$$\mathrm{CO_2}(\mathrm{t} = 0 \ldots \mathrm{n}) = \frac{1}{\mathrm{n}} \sum_{\mathrm{i}=0}^{\mathrm{n}} \mathrm{CO_2}(ti)\ \mathrm{transformed}$$

The same procedure was conducted for O_2. The transformed and filtered signals were then utilized to calculate HP based on the equations described by Lighton [15] and Gerrits et al. [9].

The detailed step-by-step procedure for a multi-chamber IC system can be shown in detail in the S2 File.

Results

Results of the recovery test

Figure 4 illustrates the minute-by-minute dynamic behavior of each chamber. All chambers exhibited similar behavior for all parameters compared to the simulated model. However, comparing the behavior between chambers throughout the assay, chambers 2 and 3 demonstrated distinct patterns and exhibited deviations from chambers 1 and 4. Chamber 2 displayed greater consistency and closely adhered to the expected behavior curve more closely than the other chambers.

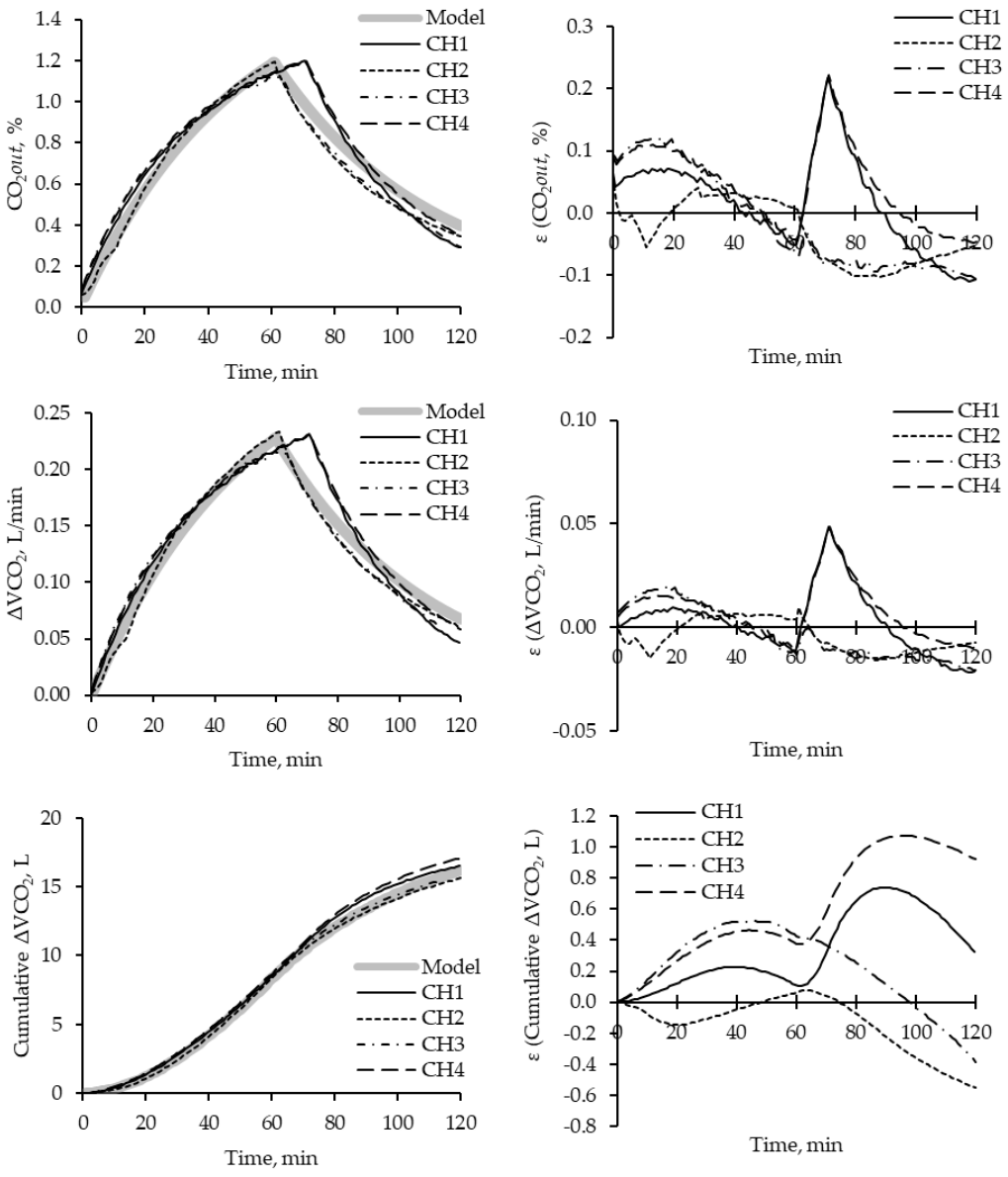

Figure 4. Dynamic (per minute) of the injection of known concentration of CO_2 injected on each chamber and description of parameters of CO_2out(*t*i), ΔVCO_2, or Cumulative ΔVCO_2, and the error calculated for each time. Each line represents the behavior of each chamber (CHn, where n refers to different chambers). The shadow line describes the expected results per unit of time according to the simulation.

The results from the injection phase revealed that chambers 2 and 3 reached their *t*inj values at approximately 60 minutes, consistent with the expected time for completing

gas injection. However, chambers 1 and 4 exhibited a delay of around 15 minutes, taking longer than anticipated.

During the injection period, all chambers exhibited slightly higher values for CO_2out concentration and ΔVCO_2 than expected. Additionally, the washing period showed greater variation in CO_2out(ti) parameters, ΔVCO_2, and Cumulative ΔVCO_2.

In general, a 1% variation in CO_2 concentration resulted in a 0.05 L/min deviation in the volumetric ΔVCO_2 above the expected value. However, this did not significantly impact the cumulative volumetric difference of CO_2, and it is unlikely to pose a problem during animal experimentation, as it only resulted in less than 1 L of ΔVCO_2 above the expected value.

Table 2 presents each chamber's recovery rates and relative standard deviation (RSD). As mentioned earlier, chambers 2 and 3 exhibited similar behavior with higher RSD(ΔVCO_2) and lower RSD (Cumulative ΔVCO_2) than chambers 1 and 4. Consequently, chambers 2 and 3 had recovery rates below 1, while chambers 1 and 4 had recovery rates above 1.

Table 2. Volumetric recovery of CO_2 (VCO_2 recovered (*ti*→120), L), recovery rate, and residual standard deviation calculated for the fractional concentration of CO_2, the volumetric difference of CO_2 (ΔVCO_2) and cumulative volumetric difference of CO_2 (Cumulative ΔVCO_2) describe for each chamber (CHn) during the recovery test.

Chamber	VCO_2 recovered (*ti*→120), L	Recovery rate	$RSD_{(\%CO2out)}$	$RSD_{(\Delta VCO2)}$	$RSD_{(Cumulative\ \Delta VCO2)}$
CH_1	16.51	1.021	0.419	0.084	0.016
CH_2	14.87	0.920	0.730	0.172	0.032
CH_3	15.09	0.933	0.476	0.115	0.020
CH_4	17.09	1.057	0.693	0.081	0.015

IC data computing and filtering

The computation of signals began by calibrating the individual signals of O_2 and CO_2 in the function of time. The analysis of gas A, with a certified concentration of 100%

N_2, 0% O_2, and 0% CO_2, revealed initial values of 0.008% O_2 and 0.004% CO_2 at the start of the measurement period and final values of 0.108% O_2 and 0.007% CO_2 at the end of the assay. Conversely, the analyzed concentration for gas B, with a certified concentration of 21% O_2 and 1% CO_2, showed initial values of 21.14% O_2 and 1.021% CO_2 at the start of the measurements and final values of 20.96% O_2 and 0.982% CO_2 at the end of the measurements (S2 File, see INPUTS sheet). These results yielded an average slope of 1.0001 and an average intercept of -0.058 for the O_2 calibration curve in the function of time. As for the CO_2 calibration curve in the function of time, an average slope of 1.004 and an average intercept of -0.006 were observed. The calibration curve was extrapolated per unit of time and is provided in the S2 File (see DATABASE sheet).

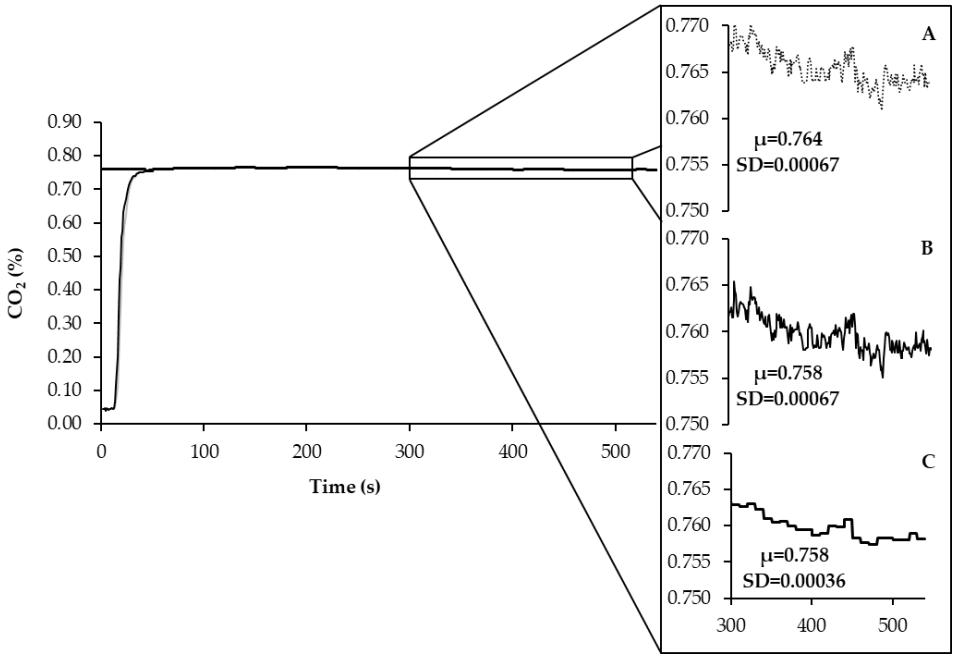

Figure 5. The curve of recorded signal (A) calibration and Bartolomew transformation applied to individual data (B) and filtered data with moving average (C) of the fractional concentration of CO_2. In the same way, was applied to O_2.

Once the calibrated signals for both gases were obtained, these were transformed and filtered to facilitate the calculation of gas exchange volumes and heat production.

These procedures were performed for individual data and expressed per minute for each chamber (S2 File, see GAS EXCHANGE sheet).

As shown in Figure 5, the average fractional concentration of CO_2, after calibration and Bartholomew transformation, exhibited a slight reduction. This reduction can be attributed to the volumetric contribution of CO_2 within the chamber, which depends on Vch, and the CO_2out measurements recorded by the analyzer. At this stage, the variation observed in the recorded and calibrated-transformed data was comparable. To further mitigate noise in the data while preserving the average of the previously calibrated-transformed data, a moving average (n=10) was applied.

Discussions

The recovery test results indicate that all chambers exhibited similar behavior compared to the simulated model in terms of dynamic parameters. However, specific differences were observed among the chambers throughout the assay, particularly in CO_2 concentration, volume injection, washing, and cumulative volume of CO_2 passing through each chamber. These differences in behavior can be attributed to variations in chamber geometry, as well as factors influencing gas exchange, such as the efficiency of gas mixing inside the chamber and the performance of the heater-cooler system [15, 16]. The presence of a temperature-controlled system inside the chamber is especially important and should be weighted and taken into consideration during calibration trials preceding each metabolic assay.

The observed variation in the delay to reach the target *t*inj among the chambers can be attributed to differences in the accuracy of filling the non-diffusion bag used in the assays [12]. As the Vinj was similar across chambers and repetitions, accurately measuring the exact volume becomes challenging and can impact the expansion of the tested gas.

During the injection period, an interesting finding was that all chambers exhibited slightly higher CO_2out concentrations and ΔVCO_2 values than expected. These variations in ΔVCO_2 are attributed to airflow (Finj or Fout), which can be calibrated using a flowmeter

to provide greater precision during animal experimentation [15]. These observations suggest the presence of some inefficiencies or measurement errors in capturing and recording gas exchange data. However, the impact of these variations on the overall cumulative volumetric difference of CO_2 was minimal, resulting in less than 1 L of ΔVCO_2 above the expected value. Therefore, these discrepancies are unlikely to significantly affect the results' accuracy or pose problems during animal experimentation.

Each chamber's recovery rates and RSD provide insights into the system's performance. As McLean and Tobin [16] recommended, the recovery rates fall within the system inefficiency range [16]. An acceptable recovery rate range of 3% to 8% (0.92 to 1.08) is considered normal.

The slight reduction observed in the average fractional concentration of CO_2 after calibration and Bartholomew transformation can be attributed to the volumetric contribution of CO_2 within the chamber, which depends on Vch, in addition to the CO_2out measurements recorded by the analyzer. The variation observed in the recorded and calibrated-transformed data was similar at this stage. To further enhance data quality by reducing noise while preserving the average of the previously calibrated-transformed data, a moving average (n=10) was applied.

The computation of IC data follows a comprehensive methodology described by Lighton [15] and Gerrits et al. [9]. This approach is applicable when working with analyzers and chambers that have undergone thorough checking and calibration. It is crucial to ensure that the chambers are defect-free or have passed a recovery test to assess their suitability. Additionally, the output signals of the analyzer should accurately represent the temporal variation observed during animal experimentation. Therefore, periodic calibration of the analyzer, recorded signal verification, and application filtering techniques are recommended to obtain coherent gas concentration values while minimizing noise. The calibration curves obtained allow for accurate transformation and subsequent filtering of the signals to calculate gas exchange volumes and heat production.

The findings from this study align with previous literature. Lighton [15] and Gerrits et al. [9] have emphasized the importance of accurate calibration and periodic verification of signals in computing IC data. Also, the present study contributes to the existing body of literature by demonstrating the importance of recovery tests in assessing the performance of chambers and the accuracy of gas exchange measurements. The variations observed among chambers highlight the need to carefully consider chamber geometry and other factors influencing gas exchange when designing experiments and interpreting results.

It is worth noting that this study has certain limitations. Using a specific animal model and experimental setup may restrict the generalizability of the results to other systems or species. Additionally, the analysis focused on CO_2 measurements, and further investigations may be necessary to evaluate the behavior of other gases or parameters and on the other IC system kinds.

Conclusion

In conclusion, this study provides valuable insights into the behavior and performance of chambers used for measuring gas exchange in animal experimentation. The observed variations among chambers and deviations from the simulated model highlight the importance of considering chamber-specific factors and calibration procedures in order to obtain accurate and reliable data.

The findings emphasize the significance of calibration and filtering techniques in improving the accuracy of indirect calorimetry (IC) measurements. This study lays the groundwork for enhancing gas exchange measurements' overall precision and reliability in future research by addressing the challenges associated with chamber behavior and data processing.

The adaptable nature of this methodology allows for its application in different IC systems and research objectives, making it a valuable tool for researchers in various fields. Moreover, the MS Excel spreadsheet provided in this study can be customized to meet specific research needs or serve as a practical teaching resource.

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CHAPTER 4 - Net energy value determination of fat and oil sources for broiler chicks

Net energy value determination of fat and oil sources for broiler chicks

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Energy, heat increment, regression method, lipids, energy value

Abstract

Energy constitutes a critical component of poultry diets, significantly influencing feed formulation due to its substantial contribution to the primary energy sources. Corn and other cereals are the primary ingredients providing a significant energy fraction in practical diets. At the same time, oilseeds are utilized for their high energy density to meet the birds' energy requirements. The metabolizable energy system (AMEn) is commonly employed to quantify the energy content of feedstuffs, though its accuracy has been subject to scrutiny. Consequently, the net energy (NE) system has been proposed as a more precise method for representing feed energy values. However, the existing NE equations face limitations in accurately determining the NE value of oils and fats. This study aimed to ascertain various energy values of oil and fat sources (NE and AME). We utilized 400 male Cobb 500 broilers, aged 11 days, for the experiment. The methodology involved a metabolic trial conducted in open-circuit respirometric chambers equipped with metabolic cages and an environmental control system, allowing for measuring oxygen consumption and carbon dioxide production to calculate heat production and excreta collection. The trial included a 4-day diet adaptation period followed by five days of data

collection. Relative energy values for soybean oil were 6914, 6440, and 4760 kcal/kg for ME, AMEn, and NE, respectively. Poultry fat exhibited energy values of 6137, 5528, and 2937 kcal/kg for ME, AMEn, and NE, respectively.

Introduction

Energy is a pivotal component in poultry diets, accounting for approximately 75% of the feed production cost. Furthermore, energy plays a crucial role in driving feed intake, subsequently influencing nutrient consumption and impacting the performance of broiler chickens (Abdollahi et al., 2018). The principal energy sources in poultry diets, after corn and cereal grains, are animal fats and vegetable oils, owing to their significant lipid composition and high caloric density compared to other ingredients (Vieira et al., 2015; Sanz et al., 2000; Randall and Latshaw, 1985).

Energy manipulation in poultry feed, commonly achieved through the addition of lipid sources such as poultry fats (PF) or soybean oil (SBO), is based on the metabolizable energy (ME) system. These lipid sources are known for their high ME concentration and well-established "extra caloric" effect, enhancing energy utilization and improving bird performance. However, it is worth noting that the ME values of these lipid sources are often added to feed formulations without considering their differential effects, leading to a wide range of energetic values, spanning from 5000 to 8900 kcal ME/kg (Song et al., 2022; CVB, 2021; Rostagno et al., 2017). This variation can be primarily attributed to the chemical characteristics, including carbon chain length, fatty acid profile, and fatty acid saturation degree (Abdollahi et al., 2018; Sanz et al., 2000; Randall and Latshaw, 1985; Vieira et al., 2015).

A key distinction between animal fats and vegetable oils lies in their saturated and unsaturated fatty acid profiles. Unsaturated sources, like SBO, are associated with higher digestibility and improved metabolic utilization of fatty acids (Sanz et al., 2000). The carbon chain length also affects the fatty acid profile and caloric potential of these lipid sources. Additionally, physical characteristics, such as viscosity, can influence passage

rates, with animal fats having higher viscosity, leading to lower digestibility (Randall and Latshaw, 1985; Song et al., 2022).

The primary difference between these lipid sources regarding ME values is their varying digestibility. The physiological explanation for this difference is that animal fats, with their high viscosity, require a higher concentration of bile salt to form micelles and transport fatty acids, resulting in lower energy availability compared to vegetable oil sources (Sanz et al., 2000; Song et al., 1999; Randall and Latshaw, 1985; Abdollahi et al., 2018). This divergence in ME values of lipid sources has been explored previously (Sanz et al., 2000; Song et al., 2022). Furthermore, the metabolic utilization of these lipid sources can vary depending on their types. Sanz et al. (2000) demonstrated that animal fats provide lower available energy for gain than vegetable sources. Diets supplemented with tallow fats in growing broiler chickens exhibited a high heat increment (Randall and Latshaw, 1985). This is attributed to the saturated fatty acids in animal fats, which have high-fat oxidation rates and lower energy retention (Sanz et al., 2000). Additionally, including lard and tallow fats in broiler chicken diets led to increased abdominal fat pad deposition, potentially resulting in birds with lower lean mass and higher rates of heat loss (Sanz et al., 2000). We hypothesize that these lipid sources can express differences in their energy values expressed on ME and NE bases due to their difference in metabolic utilization.

In poultry nutrition, the ME system is commonly used to express the energy content of diets and feed ingredients (Hill et al., 1960). However, for most diets, except for corn and soybean meal diets, the ME system has limitations as it does not consider the heat increment fraction and is inadequate for optimizing energy utilization to improve tissue deposition and body weight gain with higher efficiency. In recent years, there has been growing interest in the net energy (NE) system for expressing the energy content of poultry feeds (Kong & Adeola, 2014). The NE system considers the heat increment and has the potential benefit of expressing the energy value of ingredients with lower heat increment (Carre et al., 2014), like fat and oils. Also, this hypothesis has not been previously studied.

The metabolic energy utilization from these lipid sources can be improved by determining their NE values.

This study aimed to determine fats and oils' metabolized and net energy values and investigate their impact on energy metabolism in broiler chickens.

Materials and methods

Ethical implications

All animal procedures of utilization and management were approved by The Ethics Committee on Animal Use of the School of Agricultural and Veterinarian Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil, under protocol number nº.5401/20.

Animals and management

This study was conducted at the Poultry Science Laboratory (LAVINESP) of São Paulo State University, Jaboticabal – SP, Brazil. 400 male Cobb 500 growing broiler chickens were used in different batches (four). The birds were maintained in a climatized poultry house and allocated in grouped cages from one to 14-d-old. The facilities of each cage were equipped with linear feeders, and nipple-type drinkers supplied fresh water. During this pre-experimental period, the birds were fed *ad libitum* with a standard corn-soybean meal diet formulated to meet or exceed the nutritional requirements according to the recommendation of Rostagno et al. (2017) for growing broiler chickens for the start phases (0-14-d-old).

Treatments and experimental design

The birds were reared in four batches of 96 birds per batch. In each batch, a group of 48 birds was selected at 14-d-old, according to the same BW (±10%). The selected birds were randomly distributed on the experimental treatments. A basal diet consists of a corn-soybean meal (3100 kcal ME/kg and 22% crude protein, 1.2% Lysine SID) offered from 0 to 14-d-old.

The experimental treatment diets were supplemented with the tested ingredients (oil or fat) in gradative levels, substituted by starch formulated on a control diet (0% of fat or oil). Seven treatments consisted of control (11% of starch without oil or fat) and three increased levels of fat or oil (3, 6, 9%). Each treatment consists of six replications, with eight birds per replication.

According to the experimental protocol, the experimental diets were offered *ad libitum* from the 15 to 20-d-old and considered an adaptation period to the diets. At 21-d-old, the birds were transferred to the chambers of respirometry and adapted by 48 hours. The experimental period was from 23 to 27 for measurements and the data collection (Figure X).

Performance responses

The BW, FI, and excreta production were recorded daily during the feeding condition. The daily body weight gain (BWG) was calculated as the difference between the final BW (at the start of the fasting condition) and the initial BW when assigned to the experimental diets at the start of the adaptation period to the diets.

Indirect calorimetry method

The gas exchange measurements of oxygen consumption (VO_2) and CO_2 production (VCO_2) are done using the indirect calorimetry system described by Riveros et al. (2023). The heat production (HP) was calculated by Brower equation. That consists of

The air ingoing flow ($\mathbf{F_{in}}$), volumetric O_2 consumption (VO_2, L/b*d), and CO_2 production (VCO_2, L/b*d) were calculated following the methodology described by Lighton (2018) for an open circuit indirect calorimetry.

$$F_{in}(L/d)=(F_{out}\times\frac{(100-[O_2]_{out}-[CO_2]_{out})}{(100-[O_2]_{in}-[CO_2]_{in})}\times 1.440$$

$$VO_2(L/d)= F_{in}\times[O_2]_{in}-F_{out}\times[O_2]_{out}$$

$$VCO_2(L/d)= F_{out}\times[CO_2]_{out}-F_{in}\times[O_2]_{in}$$

Where $\mathbf{F_{out}}$ is the air outgoing flow, $[O_2]_{in}$ and $[CO_2]_{in}$ are the atmospheric gas concentrations or baseline.

The total heat production **(THP)** was calculated using the Brouwer equation (1965):

$$HP\ (kJ/d) = 16.17 \times VO_2 + 5.02 \times VCO_2$$

The nitrogen balance or retention (NR, g/bird.d) along with the nitrogen utilization efficiency (kN) was calculated derived from the nitrogen intake and nitrogen excretion using the formula:

$$NR = FI \times Nfeed - Exc \times Nexc$$

Exc denotes excreta production in dry matter (kg/bird.d).

Apparent metabolizable energy intake (MEi, kcal/bird.d) was accomplished as follows:

$$MEi = FI \times GEfee - Exc \times GEexc$$

GEfeed is the feed's gross energy content (kcal/kg feed in dry matter), and GEexc is the gross energy content in the excreta (kcal/kg excreta produced in dry matter).

The components of energy partitioning, namely ER (energy retained) and tissue deposition, were determined based on MEi and HP:

$$ER = MEi - HP$$

Energy retained as protein (ERpt) was considered the nitrogen-to-protein constant (6.25 g Protein/g N) and the protein's caloric constant (5.6 kcal/kg Protein):

$$ERpt = NR \times 6.25 \times 5.6$$

Energy retained as fat (ERfat) was obtained:

$$ERfat = ER - ERpt$$

Laboratory analyses

Feed and excreta samples were frozen at -20ºC before oven-drying at 65ºC and ground. Samples were assayed for nitrogen-corrected apparent metabolizable energy (AMEn) by determining gross energy (GE) using an adiabatic calorimeter (IKA C 2000 basic, USA) and nitrogen (N) concentration using a Kjeltech1028 distilling unit. The total excreta collection method was used to determine the metabolizable energy.

Statistical analyses

Energy metabolism parameters, MEi, HP, ER, ERpt, and ERfat, were expressed per metabolic body weight ($kg^{0.75}$). FI and BW were quantified on a per-bird-per-day basis. Meanwhile, nitrogen balance parameters were expressed in the same bases. These variables underwent analysis employing a two-way analysis of variance (ANOVA), considering the level (3, 6, and 9% of oil) and the sources as variability sources. The statistical analysis was executed using PROC GLM. In scenarios where interaction effects were observed, orthogonal contrast analyses were employed to determine the linear or quadratic tendency. Statistical significance between means was determined using the Tukey test, employing a confidence level of 95%.

Additionally, the relative energy value (on AME and NE bases) was determined through multivariate analyses, fitting to the following model:

$$Y_i = a + (b_i + \Delta b) \times X_i$$

Where Y_i represents the energy bases (AME or NE, in kcal/kg), the parameter a_i is the intercept (X_i=0 of inclusion same in both sources), the parameter b_i is the slope according to the fat or oil inclusion levels, and the Δb represent the difference of the parameters between the sources. The super index *i*th represents sources for *i*=1 for oil and *i*=2 for fat. The multivariate non-linear model was performed through PROC NLMIXED of the SAS program.

Results and discussion

The result of the parameters of the chemical composition of the analyses is shown in Table 2. A wide variation in the chemical characteristics between experimental diets was highly related to the ether extract and in minor fraction related to the starch, which released on variation of gross energy composition. The results showed that the main component that varied was the energy content due to the increasing ether extract replacing starch. This result can be explained by the difference in the caloric contribution of the ether extract (9 cal/g) and starch (4.2 cal/g), showing a high energy density of the fats and oil sources (Noblet et al., 2010; Guerrits et al., 2016).

The diets containing the poultry fat used in the experiment had a lower unsaturated to saturated fatty acid ratio than those containing soybean oil. In general, the soybean oil diets showed the highest concentration of linoleic acid and the lowest level of saturated fatty acids (calculated values from Rostagno et al., 2017).

The response parameters of each treatment are shown in Table 3. The FI, BWG, FCR, and final BW did not show an interaction between the lipid sources or level of supplementation ($P>0.05$). The lipid sources did not affect these same variables ($P>0.05$). However, the level of lipid sources supplementation level had no significant effect on the final FCR ($P>0.05$) but was seen to affect FI ($P<0.05$), BWG ($P<0.05$), and final BWG ($P<0.05$).

The energy metabolism variables are shown in Table 4. The lipid sources and level of supplementation did not influence the variables of AME intake, HP, ER, ERpt, and ERfat ($P>0.05$). Lipid supplementation levels did not significantly influence the AME intake, HP, and ERpt variables ($P>0.05$). Meanwhile, the ER and the ERfat were increased when lipid sources increased on the supplementation. These results were expected since changes in FI counteracted the differences in dietary energy concentration, leading to a similar overall energy intake and nutrients in the experimental groups. Additionally, suggestion that the FI is regulated up to satisfy the AME intake as the first driver of feed regulation (Gous et al., 1998). Similar results were described by Reece and McNaughton (1982),

who used different nutrient-density diets to feed broiler chickens reared at moderate (26·7°C) environmental temperatures. Due to the similar energy intakes, the metabolic HP and ERpt were unaffected by dietary energy density. Dietary lipid sources did not affect FI, BWG, final BW, and FCR (Table 3). This was also expected since several authors have indicated that dietary lipid source has no effect on productive characteristics as long as the ratios of energy intake to protein or energy to amino acids and other nutrients are balanced (Sibbald et al., 1962; Bartov et al., 1974; Fuller and Rendon, 1977; Hulan et al., 1984; Pinchasov and Nir, 1992), guarantying that the first limiting of FI was the energy component.

On the other hand, not significant effect of dietary fat source on total body lipid accumulation was observed ($P>0.05$). A contrary result was reported by Sanz et al., (2020), where birds receiving saturated poultry fat showed higher fat accumulation than those fed a diet containing unsaturated fats. Additionally, an interaction was observed between dietary energy level and source of lipid, in that the higher energy concentration provided by soybean oil tended to reduce body fat and the higher energy level provided by the poultry fat tended to increase ERfat. On the other hand, similar studies have yielded controversial results with regard to body fat accretion. While some reports indicate that the degree of saturation of the fat does not influence lipid accumulation (Edwards et al., 1973; Fuller and Rendon, 1977; Pinchasov and Nir, 1992), others suggest a similar effect to the present (Deaton et al., 1981; Keren-Zvi et al., 1990; Vila and Esteve-Garcia, 1996). In accordance with our findings, we showed a relationship between the lipid supplementation and the increase on the ERfat that impacted the overall ER, which can be related to the degree of saturation of the dietary fat (Sanz et al., 1999). Since energy intakes were similar in each group, the variation in ERfat may be attributable to different metabolic use of the absorbed dietary fats. A non-significant effect of dietary fat source on protein accretion was confirmed ($P>0.05$). On the other hand, Sanz et al., (2000) demonstrated a contrary result, in which higher protein accumulation occurred in broilers fed a diet rich in unsaturated fat. Su and Jones (1993) also reported less fat deposition (proportional to ERfat) and higher body lean mass in rats fed on diets containing fish oil

(rich in polyunsaturated n-3 fatty acids) than in rats fed on diets containing tallow or olive oil. In a previous researcher, was showed greater abdominal fat pad accumulation in broilers fed on diets containing saturated fat but observed no effects on weight gain (Sanz et al., 1999). It was consequently proposed that differences in the efficiency of fat use (HP) or in fat distribution might account for these differences. However, other authors suggest that differences in protein accumulation attributable to the degree of saturation of absorbed fats reflect the fact that energy from unsaturated fats can be used for metabolic purposes while energy from saturated fats is less readily used and accumulates as body lipid. Oxidation studies using labelled fats in animals (Leyton et al., 1987) and humans (Watkins et al., 1982) suggest that dietary polyunsaturated fatty acids are oxidized as fuel sources more rapidly than saturated long-chain fatty acids.

Total ER was lower in animals fed with lower lipid supplementation level, with no varying between lipids sources (soybean oil vs. poultry fat). This finding is consistent with the results of Su and Jones (1993), who reported lower energy accretion in rats fed on a diet rich in fish oil than in rats fed on diets containing tallow or olive oil. Guillaume et al. (1979) reported that a lower net energy availability of metabolizable energy was provided by a corn oil-enriched diet than by a diet containing tallow. From our experiment, it cannot be concluded which metabolic pathway is involved in differences in utilization of absorbed fatty acids with the degree of unsaturation: differential activities of enzymes involved in b-oxidation and/or selective uptake by cells or organelles are likely, depending on the tissue. Shimomura et al. (1990) reported a higher post-prandial muscle lipoprotein lipids (LPL) activity in rats fed on safflower oil than in those fed on tallow, suggesting a higher rate of muscle b-oxidation for polyunsaturated than for saturated fatty acids. Power and Newsholme (1997) found differences in the carnitine palmitoyl transferase I activity in the heart and skeletal muscle of rats fed on diets varying in the fatty acid profile, showing higher activity of this enzyme in animals fed with diets rich in menhaden oil. These findings lead to an interesting discussion in terms of ME utilization, indicating that a relative high NE may not always be of interest since it may reflect the impaired use of fatty acid residues for metabolic purposes. The incorporation of a similar fat concentration in the experimental

diets led to a higher ME concentration as the dietary unsaturated to saturated fatty acid ratio (U:S) increased. This in turn modified the energy to protein ratio. Higher FI was recorded in broilers fed diets containing poultry fat than in those fed diets which contained soybean oil. Thus, the differences in intake compensated for differences in dietary energy, leading to an overall similar energy intake (AME intake) in each experimental group. The ERfat was (numerically) lower in chicks fed soybean oil diets than in those fed poultry fat diets, even though the dietary energy density was same for both lipid sources. No effect of dietary fat saturation on ERpt was detected. However, it must be taken into account that the total amount of ingested protein was actually lower in the animals fed on diets containing soybean oil since the experimental diets were not formulated to be iso-energetic and FI decreased as the dietary energy concentration increased.

Although differences were not statistically significant, retained energy tended to be lower (numerically) in poultry fed on soybean oil diets. However, the efficiency of the use of ingested metabolizable energy for weight gain (MJ AME intake/100 g weight gain) was greater in animals fed diets containing soybean oil than in those fed poultry fat diets. This suggests that the final fate of the ingested metabolizable energy differed and was related to the degree of saturation of the dietary fatty acids.

The estimated energy value of the lipids sources was a significant fit to the non-linear mixed model on the ME ($P<0.01$), MEn ($P<0.01$), and NE ($P<0.01$) (Table 4). The relative energy value of the basal diet (11% of starch) was the same for each regression, showing that an intercept of 3128 kcal ME/kg (3122-3135), 2940 MEn/kg (2936-2944), and 2530 kcal NE/kg (2519-2567). Also, the rate of energy concentration increasing was different for each lipid source, described by the slope (b), obtaining values of 37.78 kcal per unit of soybean oil inclusion, higher than poultry fat with a rate of 30.15, suggesting that soybean oil had a higher efficiency of energy utilization due to unsaturated fatty acid composition. This difference is maintained when the energy rate increases by 34.95 kcal and 25.91 kcal per unit of soybean oil or poultry fat supplementation, respectively.

In summary, the inclusion of saturated fats in the broilers' diets led to higher fat and lower protein deposition compared with the dietary inclusion of unsaturated fats. This

effect may be only tentatively explained in terms of the different metabolic uses of the different types of fatty acids. Elucidation of the mechanisms regulating fat deposition in broiler chickens, according to the degree of saturation of dietary fats, requires further investigation. Regarding the relative energy value, the soybean oil presented 6914, 6440, and 4760 kcal/kg values for ME, MEn, and NE, respectively. On the other hand, the poultry fat presented energy values of 6137, 5528, and 2937 kcal/kg for ME. MEn and NE, respectively.

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Table 1. Feed composition and nutritional content of the basal diet.

Ingredients	**%**
Corn Gluten Meal 60% CP	11.863
Soybean Meal 45% CP	18.78
Wheat Bran - Midds	15
Corn, Grain 7.86% CP	37.349
Oil, Soybean	0
Fat, Poultry	0
Starch	11
Limestone	0.91
Dicalcium Phosphate.	1.311
Salt	0.035
Sodium Bicarbonate	0.494
L-Lysine HCl	0.411
DL-Methionine	0.155
Cl. Choline - 70%	0.1
Mineral Premix	0.1
Vitamins Premix	0.1
Inert	2.392
Total	100
Calculated nutritional information	
ME, kcal/kg	2958
Crude Protein, %	21.5
Total Ca, %	0.76
AvP, %	0.38
K, %	0.664
Na, %	0.16
Cl, %	0.16
Lysina SID, %	1.028
Methionine SID, %	0.493
Methionine + Cystine SID, %	0.8
Threonine SID, %	0.673
Tryptophan SID, %	0.193
Arginine SID, %	1.07

Table 2. Energy and proximal analyses composition of the experimental diets

Sources	Basal diet	Soybean oil			Poultry fat		
Level (%)		3	6	9	3	6	9
Crude protein (%)	21.22	20.98	21.4	21.06	21.01	20.87	20.31
Ether extract (%)	2.53	5.52	8.51	11.5	5.40	8.33	10.97
Starch (%)	40.25	37.62	34.98	31.98	37.91	35.9	32.81
Ash (%)	5.40	5.70	5.20	5.00	5.50	5.10	5.50
Gross energy (kcal/kg DM feed)	3820	3988	4156	4324	3986	4153	4319

Table 3. Performance response of growing broiler chickens from 14 to 22-d-old feed with different soybean oil and poultry fat levels.

Sources	Level	FI (g/b.d)	BWG (g/b.d)	FCR	Final BW (kg)
Basal diet	**0**	147	96	1.521	1.382
Soybean oil	**3**	142	95	1.522	1.378
	6	132	87	1.556	1.336
	9	127	84	1.583	1.306
Poultry fat	**3**	147	97	1.529	1.362
	6	128	84	1.587	1.354
	9	125	85	1.485	1.300
SEM					
Sources	**Soybean oil**	134	89	1.554	1.340
	Poultry fat	133	89	1.533	1.338
Level	**3**	145[a]	96[a]	1.525	1.370[a]
	6	130[b]	85[b]	1.572	1.345[ab]
	9	126[b]	85[b]	1.534	1.303[b]
Probability					
Souces		0.828	0.962	0.600	0.922
Level		<0.001	0.013	0.582	0.012
Interaction		0.530	0.758	0.365	0.706

Table 4. Energy partitioning components (expressed per unit of metabolic body weight) of growing broiler chickens from 14 to 22-d-old feed with different soybean oil and poultry fat levels.

Sources	Level	AME intake (kcal/kg$^{0.75}$.d)	HP (kcal/kg$^{0.75}$.d)	ER (kcal/kg$^{0.75}$.d)	ERpt (kcal/kg$^{0.75}$.d)	ERfat (kcal/kg$^{0.75}$.d)
Basal diet	**0**	369	169	200	97	102
Soybean oil	**3**	359	179	180	98	81^{b}
	6	363	165	198	94	104^{ab}
	9	361	159	202	92	111^{ab}
Poultry fat	**3**	350	173	176	97	79^{b}
	6	340	171	169	96	73^{b}
	9	367	151	216	90	126^{a}
SEM						
Sources	**Soybean oil**	361	168	193	95	99
	Poultry fat	352	165	187	94	93
Level	**3**	356	169	187^{ab}	96	92^{ab}
	6	349	175	174^{b}	97	77^{b}
	9	364	155	209^{a}	91	118^{a}
Probability						
Souces		0.139	0.779	0.501	0.857	0.685
Level		0.161	0.157	0.045	0.245	0.079
Interaction		0.331	0.651	0.468	0.725	0.051

Table 5. Parameter of the regression of the AME and NE feed values in function of the soybean oil or poultry fat levels.

Sources	Equation			Probability				Estimated value
				Regression	Sources	Level	Interaction	
Soybean oil	AME (kcal/kg)	=	3135 + 37.78 level	<0.001	0.628	<0.001	0.004	6914
Poultry fat	AME (kcal/kg)	=	3122 + 30.15 level					6137
Soybean oil	AMEn (kcal/kg)	=	2944 + 34.95 level	<0.001	0.525	<0.001	0.054	6440
Poultry fat	AMEn (kcal/kg)	=	2936 + 25.91 level					5528
Soybean oil	NE (kcal/kg)	=	2519 + 22.4 level	0.0315	0.636	0.077	0.298	4760
Poultry fat	NE (kcal/kg)	=	2567 + 19.7 level					4437

CHAPTER 5 - Net energy prediction of feed for broiler chickens

Net energy prediction of feed for broiler chickens

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Abstract

Net energy (NE) in poultry is regarded as a more precise energy system compared to conventional apparent metabolizable energy corrected by nitrogen balance (AMEn) due to its ability to consider the energy lost as heat increment from nutrient metabolism that averages 25% of AMEn and is variable with feed characteristics. However, unlike pigs, NE still needs to be implemented in poultry nutrition. This study aimed to measure the NE value of 48 diets highly variable in terms of chemical composition (28 to 44% for starch; 18 to 29% for crude protein (CP), and 5.6 to 12% for fat (EE)) to establish NE prediction equations for broiler feeds. Diets were prepared with 16 major ingredients (wheat, corn, poultry byproduct meal, peanut meal, meat, and bone meal, soybean meal, cottonseed meal, canola meal, wheat brand, rice brand, millet, sorghum, starch, poultry fat, and soybean oil) at various and independent inclusion levels in order to calculate robust prediction equations between NE content and chemical constituents or levels of ingredients. All diets was formulated with same AMEn to SID Lys rate. The diets were fed to male Cobb 500 broilers kept in open-circuit respiratory chambers from 21 to 28 days of age (8 birds per cage) after five days of diets adaptation and to the chambers, diet AME, AMEn content and heat production were determined and calculation of diet NE value. The trial was planned weekly with six diets measured in each batch (one per chamber). Each diet was randomly distributed in the chambers for each batch and tested in 4 replicates. Performance of birds (114 g/d BW gain on average), diet AMEn (3266 kcal/kg dry matter on average; range: 2840 to 3671), and AMEn/GE (71.4% on average) were as expected. The NE/AMEn ratio averaged 75.8% (range: 70.0 to 80.2%) and increased with EE and decreased with CP in the feed. Accordingly, diet NE value was positively related to AME and EE contents and negatively to CP level. Starch had no significant impact on NE prediction. The NE equations (NE (kcal/kg Dry Matter) = 0.82 × AMEn – 10.04 × CP + 14.8 × EE) generated in the current study show a good agreement with previous studies

on poultry and pigs and were also able to predict the NE value of the ingredients of our trial. This study further highlights the importance of the NE approach in broilers.

Introduction

Optimization is a key interest in the feed formulation of poultry diets, referring to efficiently using resources while minimizing waste. Proper application of optimization strategies directly impacts profitability. As energy is one of the main components considered during diet formulation, it significantly influences diet optimization (Azebedo et al., 2021). Efficient energy utilization is related to matching dietary energy supply as closely as possible to the animal's requirements to reach its production potential (Emmans, 1994). This approach also involves the accuracy of the energy system in predicting the bird's response. However, in developing a system, both the animal's requirements and the feed's energy value must be considered, with the latter being the focus of this study.

Traditionally, the apparent metabolizable energy (AME) system and its nitrogen-corrected expression (AMEn) are predominantly used in poultry diet formulation. This system has performed well, effectively optimizing poultry diets since the current practical feed formulas are based on corn and soybean meal. These ingredients show low variation in nutritional composition and a nutritional profile (mainly digestible amino acids) close to poultry requirements with high efficiency of utilization (around 76%). However, this system is challenged when incorporating non-traditional or alternative ingredients (e.g., wheat, sorghum, canola meal, DDGs, etc), which may vary in nutritional composition, affecting the efficiency of utilization (Swick et al., 2014), and these system not fully account the energy lost as heat or heat increment (HI) during metabolism (Carre et al., 2014; Fraps and Carlyle 1942; de Groote 1974; Swick et al., 2013). Including these ingredients in the feed can represent variations of around 20 to 30% of HI (Carré et al., 2014; Choct, 1999; Wu et al., 2019). This variation represents an opportunity for nutritionists to implement strategies to reduce HI and promote the inclusion of alternative ingredients aiming to reduce feeding costs. In this context, the development of the net energy (NE) system has gained interest among researchers as it could represent the "real" use of energy since it

accounting the HI fraction and supplies energy for maintenance (fasting heat production, FHP) and energy retained in body weight, apparently offering a favorable tool to improve the optimization of feed.

The NE system has been widely used in other species, such as dairy cattle and pigs, especially the latter (Carré et al., 2014; De Groote, 1974; Noblet et al., 2023a; Van der Klis and Kwakernaak, 2008). However, its application in poultry was limited by the scarcity of information and studies conducted. Although the NE system in poultry was explored before the 90s (De Groote, 1974), it only gained interest recently (Carre et al., 2014; Wu et al., 2019; Tay-Zar et al., 2023). The fluctuating energy costs of feed ingredients linked to biofuel production and non-agricultural commodities have made energy formulation techniques more flexible due to the increase of feedstuffs coming from agroindustrial byproducts and other alternative sources availability (Yu et al., 2006; Harri et al., 2009).

The development of the NE system for poultry in most current studies was facilitated by adapting the indirect calorimetry method for measuring metabolic heat production, as the interest lies in quantifying the heat increment fraction of feeds to calculate their respective NE values (Wu et al., 2019; Barzegar et al., 2014; Tay-Zar et al., 2023; Riveros et al., 2023). Additionally, based on the premise that energy utilization results from the metabolic use of nutrients, predicting the energy value of feeds from their nutritional composition seems feasible. NE feed prediction equations can be developed using statistical tools and an understanding of poultry metabolism. Various authors have proposed different equations, such as Carre et al. (2014), who proposed sophisticated NE equations based on digestible nutrients. On the other hand, Wu et al. (2019) and the more recent Tay-Zar et al. (2023) propose simpler and more practical equations based on chemical composition. Several factors may be involved in "better" prediction equations and their accuracy, but undoubtedly, developing an NE system for poultry should be constructive among various experiences.

This study aims to develop a reliable NE prediction equation for broilers, addressing the gaps in previous research and conflicting literature data. The focus is on generating

accurate NE prediction equations from different feedstuffs chemical compositions and ME values.

Materials and methods

Ethics and animal housing

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 (CEUA, Protocol no 5401/20).

A total of 1200 on-day-old male broiler chickens were periodically obtained from a local hatchery (Pluma, Valinhos, SP-Brazil), and allocated in groups of ten birds per cage (0.8 m x 0.9 m x 0.5 m) with environmentally controlled poultry house facilitating the temperature adjustment according to the Genetic Guideline (Cobb Vantress, 2020). Each cage was equipped with linear feeders and nipple-type drinkers.

Protocol and experimental design

The trial was divided into pre-experimental (1-14-old-days), diet adaptation periods (15-20-old-days), and experimental periods (21-28-old-days). During the pre-experimental period, birds were fed a standard commercial diet based on corn and soybean meal, formulated to meet the nutritional requirements (Cobb Vantress, 2020). Water and feed were provided *ad libitum* throughout the entire experiment period. The lighting program adopted was to provide 24 h of light for the pre-experimental period and 16L:8D during the experimental period.

During the adaptation period, the birds were randomly distributed in the cages, guaranteeing a similar average body weight between experimental units. After the adaptation period, the birds were transferred to the respirometry laboratory and acclimatized in the chambers for two days. Afterward, the data collection began under feeding conditions with the respective experiment diet for four days. On the last day, the

feed was withdrawn, and the gas exchange measurements were conducted under fasting conditions for 24 hours.

At the start of the adaptation period, for each trial, a group of 48 birds was selected from a population of 100 birds according to their body weight (450±100 g), guaranteeing a uniformity of around 90%. For each trial, the birds were randomly distributed in six chambers of respirometry in groups of eight birds per chamber, where each chamber consisted of an experimental unit for a respective experimental diet. The trials with the same group of diets were replicated three or four times (Table 1) in a consecutive order.

Criteria or feed formulation and experimental Diets

A total of 48 experimental diets were formulated to have a wide variation in the nutrient composition and AMEn value (Table 1). The experimental diets were performed using traditional and non-traditional ingredients to provide flexibility in the nutritional composition and allow a wide range of energy intake to be evaluated between experimental diets. The variation in the nutritional composition of the diets (calculated values), on a dry matter basis, ranged from 2562 to 3267 kcal/kg of AMEn, 16.23 to 24.59% crude protein (CP), 4.92 to 11.41% ether extract (EE), 11.57 to 18.52% NDF and 24.71 to 39.64% starch (Table 1). The high variation between dietary nutrients was established to facilitate the application of the statistical procedure to achieve either no or minimal correlation among predictors (nutrients).

The diet formulation was made based on the energy and nutritional composition of the ingredients as described in the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017). Additionally, crystalline amino acids were supplemented to the diets to correct the balance between AME and amino acids, thus ensuring that the variation in feed intake did not limit amino acid intake.

Indirect calorimetry protocol and gas exchange measurement

During the experimental period, the body weight and the feed intake were recorded daily, and an overall FI and BW were calculated for the adaptation and experimental period and expressed daily.

The indirect calorimetry system used was the same as Riveros et al. (2023) described. That consists of the gas exchange measurements of oxygen consumption (VO2) and CO2 production (VCO2) to calculate the heat production (HP) through Brower fundamental equation.

The air ingoing flow (**F_{in}**), volumetric O_2 consumption (VO_2, L/b*d), and CO_2 production (VCO_2, L/b*d) were calculated following the methodology described by Lighton (2018) for an open circuit indirect calorimetry.

$$F_{in}(L/d)=(F_{out}\times \frac{(100-[O_2]_{out}-[CO_2]_{out})}{(100-[O_2]_{in}-[CO_2]_{in})}\times 1.440$$

$$VO_2(L/d)= F_{in}\times[O_2]_{in}-F_{out}\times[O_2]_{out}$$

$$VCO_2(L/d)= F_{out}\times[CO_2]_{out}-F_{in}\times[O_2]_{in}$$

Where **F_{out}** is the air outgoing flow, $[O_2]_{in}$ and $[CO_2]_{in}$ are the atmospheric gas concentrations or baseline.

The total heat production **(THP)** and fasting heat production **(FHP)** were calculated using the Brouwer equation (1965):

$$HP\ (kJ/d)\ =16.17\times VO_2+5.02\times VCO_2$$

The FHP was determined considering when the heat production reached a plateau during the fasting period and under the criteria of the RQ close to 0.7. A conditional criterion was established to determine the FHP as a procedure to compute the data of FHP on each trial, as described in the computing gas exchange data detailed in Riveros et al., (2023).

Calculation of the apparent metabolizable energy intake (MEintake, kcal/bird.d), and the other energy metabolism parameters were calculated as follows:

$$MEintake = FI \times GE_{feed} - Exc \times GE_{exc}$$

$$ER = MEintake - HP$$

$$HI = HP - FHP$$

$$NEintake = ER + FHP$$

Where, GEfeed is the gross energy of feed (kcal/kg feed in dry matter), GEexc represents the gross energy in the excreta (kcal/kg excreta produced in dry matter), ER is the energy retained, HI is the heat increment, and the NEintake represent the net energy intakes (kcal/$kg^{0.75}$/d)

Chemical Analysis and Calculations

The experimental feed and excreta were collected, sampled, and homogenized for further laboratory analysis. All samples were subjected to dry matter content determination at 105˚C for 16 hours. The nitrogen content of the samples was measured using the Kjeldahl method (AOAC, 2005), and the crude protein content was calculated using the coefficient of 6.25 g of crude protein per g of nitrogen. The ether extract of the feeds and excreta was determined through extraction with organic solvent (ANKOM Technology® apparatus). The gross energy of feed and excreta was determined through an adiabatic calorimeter (IKA C 2000 basic, USA).

The AME was determined using the total excreta collection (Bourdillon et al., 1990). The AME values were converted to AMEn using the nitrogen balance and the coefficient 8.22 kcal/g of N as a correction factor (Hill and Anderson, 1958) for zero N retention in the body.

Statistical analysis

The performance and energy balance data were analyzed using the software SAS (SAS Systems Inc., Cary, NC). Pearson correlation analysis was performed using the

PROC CORR procedure between nutrients and energy. The formulation criteria of experimental diets were conducted independently to ensure the absence or minimal correlation between nutrients and energy and to maintain a wide composition variation.

The nutritional composition and energy value were subject to multiple regression analyses considering the NE, AME, and AMEn values as dependent variables and the nutritional composition and AME and AMEn as predictors. The statistical analyses were conducted through the PROC REG procedure, using the stepwise method to determine the significance of the predictors. The regression model was as follows:

$$y = \beta 0 + \beta 1x1 + \beta 2x2 + \ ... + \beta nxn + \varepsilon$$

Where x1, x2 ... xn are the predictor nutrients of the equations, β1, β2, …βn are the coefficients of the respective nutrients, and ε is the corresponding random error of the model.

The significance level for variables inclusion or exclusion in the model was established at $P<0.05$. Additionally, the root mean square of errors (RSD) was calculated to evaluate the developed equations:

$$RSD = \sqrt{\frac{(Y_{obs} - Y_{pred})^2}{n}}$$

Results and discussions

Criteria, formulation, and experimental diets composition

In the formulation of experimental diets for this study, a diverse array of ingredients commonly utilized in commercial broiler chicken feeds, comprising both conventional and unconventional feedstuffs, was selected (Table 1). The formulation adhered to the following criteria: (1) Ingredient inclusion levels were capped according to the recommendations of Rostagno et al. (2017). (2) The aim was to standardize the ratio of AMEn to digestible lysine (3.55 g Lys SID/Mcal AMEn ± 0.2) across all diets, maintaining a balanced protein profile achieved through the addition of synthetic amino acids; (3)

Mineral requirements (for calcium and available phosphorus) and electrolyte balance (chloride, potassium, and sodium) were uniformly met across diets through the addition of limestone, dicalcium phosphate, salt, and sodium bicarbonate, in alignment with the nutritional levels recommended by Rostagno et al. (2017).

The study noted a predominant inclusion of poultry by-product meal across experimental diets (n=32), attributed to its significant protein and energy contributions even at relatively low inclusion rates (maximum of 8.17%). In contrast, cottonseed meal featured less prominently (n=5) due to its recommended maximum inclusion limit of 10% in poultry diets, as Rostagno et al. (2017) advised. This broad ingredient spectrum enhances diet formulation flexibility and challenges system development by incorporating diverse nutrient sources.

However, the success in formulating predictive equations for NE depends not solely on the variety and number of ingredients or experimental diets. That is exemplified by studies such as Wu et al. (2019), which utilized a similar ingredient array (n=17) with comparable formulation principles, and Tay-Zar et al. (2023), which incorporated 13 ingredients, showcasing similar nutritional variation. On the other hand, Carre et al. (2014) employed a more extensive range of diets (n=30) without accounting for the Lys SID to AMEn ratio, resulting in greater dietary composition variation. These instances highlight the critical role of nutritional composition in experimental diet formulation in developing NE equations.

Attention was particularly focused on nutritional composition during diet formulation, with calculated values for each ingredient based on the Brazilian Tables (Rostagno et al., 2017) and detailed proximate and energy composition (Table 2). Observed and analyzed values for CP, EE, and NDF were closely aligned, validating the reference values used in the formulation. However, Starch analysis revealed slightly higher values (mean 34.7 vs. 38.5, analyzed vs. calculated, respectively) impacting the OM. Starch content determination in feeds can vary by method, including polarimetric and enzymatic hydrolysis, with inherent methodological variations that can lead to 5-10% discrepancies between laboratories and protocols, as the European Committee for

Standardization (CEN) noted. These variations, influenced by non-starch polysaccharides (NPS) and carbohydrate structures in the samples, affect enzymatic determination, as Pirgozliev et al. (2003) discussed. Feed energy value is influenced by starch content, given its primary role as an energy source and its high prevalence in poultry feeds, affecting digestibility as explored by Zeafarian et al. (2015). Variations in starch and OM trends suggest the presence of other carbohydrate types that could influence the digestion of other nutrients, as indicated by Abdel-Hafeez et al. (2017). As starch and other carbohydrates are the main energy sources, the variation on this can be influenced by the energy determination expressed on different bases, which can be reflected in the energetic contribution of the nutrients that will be discussed afterward.

Variations in the energy composition of feeds, as indicated by analyzed values for GE, AME, AMEn, and NE, were observed, exhibiting coefficients of variation of 2.08%, 6.22%, 6.36%, and 7.67%, respectively. These variations were broadly consistent with findings by Tay-Zar et al. (2023) and slightly higher than those reported by Wu et al. (2019), highlighting the critical role of nutrient digestibility and metabolic efficiency in energy utilization. The ratios of AME to GE and AMEn to GE, which serve as indicators of dietary metabolic utilization, displayed considerable variability, with CVs of 4.79% and 5.02% and average values of 76% and 71%, respectively. Moreover, energy utilization efficiency, as depicted by the ratios of NE to AME and NE to AMEn, averaged 76% and 80%, with CVs of 3.35% and 2.50%, respectively. These results are in alignment with the metabolic efficiency observed in typical poultry diets, corroborating findings by Swick et al. (2014) for diets based on corn and soybean meal, which demonstrated a similar utilization efficiency of approximately 75%. This analysis underscores the inherent variability in feed energy composition and the resultant impact on metabolic energy utilization. It also emphasizes the importance of accurately assessing energy values and efficiency rates to optimize dietary formulations and enhance the performance of poultry chickens.

The study examined the correlations between major nutrients and energy (Table 3). This analysis was crucial because the energy equation based on nutritional

composition must be statistically constructed to mitigate the collinearity effect; otherwise, certain variables may disproportionately influence the developed equation (Kutner et al., 2004). The results revealed that EE and CP were not significantly correlated ($P>0.05$), and similarly, EE and starch did not exhibit a significant correlation ($P<0.05$). Conversely, a negative correlation was observed between CP and starch ($P<0.05$), indicating that a reduction in energy from CP might be compensated by an increase in starch levels in the diets. This pattern aligns with findings reported by Wu et al. (2019) and Tay-Zar et al. (2023). Furthermore, Barzegar et al. (2020) reported a negative correlation between EE and starch in the diets of laying hens, suggesting that, alongside CP, EE may also need to be balanced by starch inclusion to offset energy contributions.

Overall, broilers utilized 76.0% of dietary GE as AME, which reduced to 71.4% when nitrogen correction was applied, as indicated by the AMEn/GE ratio. The efficiency of converting AME to NE averaged 75.8%, and for NE/AMEn, it was 80.2%. The increased efficiency observed with AMEn calculations can be attributed to nitrogen correction enhancing the value of energy utilization. This is because the energy portion corresponding to uric acid excretion is reattributed to the feed's energy value, assuming complete dietary nitrogen utilization. One hypothesis we propose, which explains the effective functioning of the AMEn system in current poultry nutrition, is based on this assumption. Nitrogen correction might inadvertently enhance energy utilization. However, this could become a disadvantage in novel nutritional strategies that involve reducing crude protein levels, where the NE system could provide a more accurate representation of energy utilization.

Birds response and energy metabolism parameters

The growth performance and energy balance parameters of the birds are detailed in Table 4. The broiler chickens, sourced from the Cobb 500 strain and procured from the same hatchery albeit in separate batches, were utilized to facilitate the execution of the experiment. According to the Genetic Guidelines provided by Cobb Vantress (2018), the target average body weight (BW) for birds at 28 days of age is 1.28 kg. The minimum BW observed in this study surpassed this benchmark, indicating that the birds were in optimal

conditions to realize their genetic potential, with the experimental diets being the primary variable affecting their performance and energy utilization.

To ensure consistent performance and responses across trials, careful selection was implemented at the commencement of each trial to standardize bird uniformity across batches. Despite originating from the same hatchery, the birds were received into the trials on different dates, with an initial allocation of 100 birds per batch. At 21-old-d, a subset of 48 birds, maintaining uniform BW across all batches, was selected. The diets were then randomly assigned across six chambers sequentially to maintain feed quality. As highlighted by Ta-Zar et al. (2023), managing uniformity across batches is crucial, and randomization serves to minimize variability factors other than diet, which the availability of chambers may influence. Furthermore, FI, following the energy content of the feed on both AMEn and NE bases, was recorded, although the data are not presented. The FCR adhered to the standards recommended by Cobb Vantress (2018). The energy intake patterns were consistent with the findings of previous studies (Carre et al., 2014; Cerrate et al., 2019; Caldas et al., 2023).

A significant correlation was observed between dietary composition and energy intake values for AME and NE; lower energy intakes were associated with high-CP diets, while higher energy intakes corresponded to low-CP and/or high-fat diets. Variability among diets in terms of FHP, HP, and HI was minimal, with approximately 50% of AME intake being expended as HP. These findings agree with those Riveros et al. (2023) reported for roosters and Sakomura et al. (2004) for ad libitum-fed growing chickens. The slight variations in FHP between diets were, on average, consistent with Sakomura et al. (2004) and Martinez et al. (2022), albeit slightly lower than the values reported by Noblet et al. (2015), who estimated 420 kJ/kg^0.70/day (equivalent to 102 kcal/kg^0.75/day). The discussion surrounding maintenance energy requirements and the allometric determination of maintenance parameters based on an empirical fit (a*BW^b), remains a topic of debate among researchers. In this study, individual FHP values corresponding to each diet were utilized to refine NE estimations, leveraging the same equipment to measure both HP and FHP in the same cohort of animals.

The dietary changes and their negligible impact on HP and total retained energy (RE) were primarily influenced by CP content, with high-CP diets leading to increased HP values and decreased RE, contrary to the effects of high-fat diets. The impact of CP-rich diets on HP directly influenced HI, given the lower variability in FHP. These outcomes are consistent with findings in other species, such as broiler breeders (Teofilo et al., 2023) and studies utilizing similar calorimetric systems (Riveros et al., 2023).

Respiratory quotient (RQ) values, calculated from the volumetric rates of oxygen consumption and carbon dioxide production, were close to 1, indicative of ad libitum feeding conditions, as Guerrits et al. (2017) supported. Diets high in EE were associated with slightly lower RQ values (around 0.95), while diets rich in starch yielded higher RQ values (approximately 1.02) (data are not provided).

Energy utilization and nutrients

The observed variations in the metabolic utilization of diets and the efficiency of converting AME to NE underline the differential impact of various nutrients on energy efficiency. This underscores the importance of quantifying the contributions of major energy-yielding nutrients to the overall energy supply. Such an analysis was conducted using linear regression on the mean values obtained from 48 diets (Table 5). The regression coefficients for CP, EE, and starch provide insights into the contribution of each nutrient to energy systems such as GE, AME, and NE. Specifically, the contributions of CP, EE, starch, and the undetermined fraction (which includes free-sugars and other components of OM) to GE were 53%, 94%, 38%, and 32%, respectively. These findings are consistent with the analytical determinations of macronutrient energy content as reported by Guerrits et al. (2017). Notably, the starch content observed in this study was marginally lower than the figures reported by Noblet et al. (2010) and Wu et al. (2019), possibly due to methodological and laboratory variations in starch determination.

The analysis revealed a decreased energetic contribution from CP when expressed in terms of AME and AMEn. This decrease is attributable to the reduced metabolic utilization of CP from the digested fraction to the excreted fraction, a phenomenon

particularly pronounced when considering AMEn (Emmans, 1994). Conversely, EE maintained a relatively stable energetic contribution across both AME and AMEn calculations. Wu et al. (2019) noted an increased metabolic utilization of EE, with parameters exceeding 100 when expressed in terms of AME and AMEn, reflecting the variability in the nutritional composition of the experimental diets (Swick et al., 2014).

Moreover, all nutrients (CP, EE, Starch, and the Undetermined fraction) exhibited decreased efficiency of utilization when their contributions were expressed on NE basis. This reduction is attributed to energy losses as HI during the digestion and absorption processes (van Milgen et al., 2010). Notably, CP was identified as the primary nutrient, experiencing a significant drop in utilization efficiency, approximately 60%, highlighting its substantial energy losses during metabolism.

Equations to predict the AME, AMEn and NE

Consistent with the observed high correlations between AME values and the content of CP and EE (Table 3), as well as the significant impact of EE and starch outlined in Table 5, CP, EE, and starch emerged as the foremost predictors of AME, AMEn and NE. These predictors were effective with or without the inclusion of an intercept component, also revealing differences in the residuals. Notably, EE demonstrated the highest coefficient value within each AME predictive model that incorporated CP and starch, aligning with expectations. In the stepwise regression model for predicting NE, AME was identified as the initial predictor. This finding aligns with the strong correlations observed between NE and the indicators for CP and EE, suggesting that the most accurate NE predictions are achieved by including CP, EE, and AME content. The coefficient for AME in these predictive equations was approximately 0.82, closely mirroring the conversion efficiency of starch-derived AME to NE (82%), thereby negating the significance of starch in any NE predictive model. While CP had a detrimental effect on NE values, EE positively influenced the NE content of the diets.

This research aimed to assess the NE values and the efficiency of converting AME to NE across 48 nutritionally balanced diets with diverse chemical compositions. The

primary objective was to develop a set of equations capable of predicting the AME, AMEn, and NE values of diets and raw ingredients based on their chemical compositions, utilizing a straightforward and reliable method. The most representative equations for NE prediction derived from this study are: NE (kcal/kg Dry Matter) = 0.815 × AME – 12.8 × CP + 14.76 × EE, and NE (kcal/kg Dry Matter) = 0.82 × AMEn – 10.04 × CP + 14.8 × EE. These equations are applicable to both complete feeds and individual ingredients, representing significant advancement compared to recent studies in poultry nutrition. The coefficients within these equations underscore the dominant contribution of the AME value to the NE value over the chemical composition, highlighting the critical need for an accurate AME system to ensure precise NE value predictions.

Given the limited number of studies on broiler feed NE values that offer comparable methodologies, conceptual frameworks, and robust trial designs, it is prudent to synthesize these findings and propose a more generalized set of NE prediction equations. This approach would enhance the predictability and applicability of NE values in poultry nutrition, facilitating more efficient and targeted feed formulation strategies.

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Table 1. Composition of experimental diets.

Ingredients	n	Max	Min	Avg.
Corn, Gluten Meal 60% CP	8	5.77	0.7	3.2
Poultry Byproduct Meal	32	8.17	0.5	3.62
Peanut Meal	6	12	0.9	4.65
Meat and Bone Meal, 48%	19	3.54	0.1	1.99
Soybean Meal 45% CP	22	30.5	1.1	14
Cottonseed Meal 39% CP	5	9.97	0	5.23
Canola Meal	28	12	0.3	6.98
Wheat Bran - Midds	10	17.9	0	7.79
Rice Bran	21	15	0	8.84
Millet, Grain	29	32.8	0.8	13.8
Wheat, Grain	14	35	1.2	21.2
Sorghum Grain, High Tannin	10	35	0	21.5
Corn, Grain 7.86% CP	13	62	0.5	15.3
Oil, Soybean	23	9	0.2	3.9
Fat, Poultry	8	6.36	0	3.68
Starch	9	15	0.4	8.56
Inert	12	4.93	0.9	3.29
Limestone	27	0.97	0	0.57
Dicalcium Phosph.	38	1.17	0.1	0.57
Salt	38	0.29	0	0.1
Sodium Bicarbonate	48	0.68	0.2	0.53
L-Lysine HCl	48	0.67	0.1	0.41
DL-Methionine	48	0.27	0.1	0.18
L-threonine	47	0.2	0.1	0.15
L-Tryptophan	26	0.05	0	0.01
Arginine	35	0.31	0	0.11
Valine	45	0.24	0	0.09
Cl. Choline - 70%	48	0.1	0.1	0.1
Mineral Premix	48	0.1	0.1	0.1
Vitamins Premix	48	0.1	0.1	0.1

Table 2. Calculated and analyzed the energy and nutritional composition of experimental diets.

	Calculated			Analyzed		
Variable	Max	Min	Avg.	Max	Min	Avg.
Crude protein, %	28.59	16.23	22.12	29.1	18.0	22.86
Ether extract, %	11.41	4.92	8.32	12.3	5.60	9.16
Starch, %	39.64	24.71	34.66	44.4	27.5	38.53
NDF, %	18.52	11.57	14.3	20.6	8.10	13.679
Organic matter, %	87.24	80.78	83.78	93.9	91.9	92.98
GE	-	-	-	4798	4416	4600
AME	3877	2998	3398	3884	3023	3459
AMEn	3537	3035	3272	3671	2840	3266
NE	-	-	-	3065	2260	2624
AME/GE	-	-	-	83.4	68.8	76.0
AMEn/GE	-	-	-	78.7	64.3	71.4
NE/AMEn	-	-	-	80.2	70.0	75.8
NE/AME	-	-	-	83.7	75.7	80.2

Table 3. Pearson correlation analyses between nutrients and energy of the experimental diets.

Nutrients	CP		EE		Starch		CF		NDF		GE		AME	
EE	-0.319	NS												
Starch	-0.603	<0.01	-0.164	NS										
CF	0.038	NS	-0.128	NS	-0.485	<0.05								
NDF	0.173	NS	-0.226	NS	-0.548	<0.01	0.760	<0.01						
GE	-0.056	NS	0.931	<0.001	-0.287	NS	-0.130	NS	-0.120	NS				
AME	-0.380	<0.05	0.768	<0.001	0.312	NS	-0.575	<0.01	-0.690	<0.001	0.690	<0.001		
NE	-0.451	<0.05	0.772	<0.001	0.349	NS	-0.533	<0.01	-0.673	<0.001	0.671	<0.001	0.989	<0.001

Table 4. Effect of the diet composition on the experimental diets' growth performance, energy balance, energy value, and energy utilization.

Parameter	Avg.	Max	Min
FI, g/bird/d	134	153	118
BWG, g/bird/d	114	124	101
FCR	1.17	1.25	1.10
BW, kg/bird	1.34	1.40	1.26
AME intake, kcal/kg0.75/d	357	384	308
NE intake, kcal/kg0.75/d	282	334	235
HP, kcal/kg$^{0.75}$/d	166	203	136
HI, kcal/kg$^{0.75}$/d	74	111	44
FHP, kcal/kg0.75/d	93	102	86
ER, kcal/kg0.75/d	190	242	143
RQ	1.00	1.05	0.92

Table 5. The energy contribution of the yielding nutrients (%DM basis) to the experimental diets' GE, AME, AMEn, and NE.

Variable	CP	EE	Starch	Und (OM-CP-EE-Starch)	Rsq
GE (kcal/kg)	53.9	93.9	38.2	39.1	99.9
AME (kcal/kg)	33.7	93.0	42.2	26.1	102.8
AMEn (kcal/kg)	28.1	94.1	42.2	30.6	89.3
NE (kcal/kg)	17.0	89.1	32.8	28.7	34.1

Table 6. Prediction equations for AME. AMEn and NE of broiler diets from the chemical composition (%DM basis).

Variable	Intercept	AME		AMEn		CP	EE	Starch	Und (OM-CP-EE-Starch)	RSD
AME (kcal/kg)	523 ±158 0.002					31.2 ±3.23 <.0001	74.9 ±5.67 <.0001	36.8 ±5.67 <.0001		67
AME (kcal/kg)						32.8 ±2.32 <.0001	70.3 ±5.34 <.0001	42.2 ±1.55 <.0001	26.1 ±1.73 <.0001	65
AMEn (kcal/kg)	-335 ±260 0.209					47.1 ±11.1 <.0001	77.6 ±9.65 <.0001	45.8 ±4.4 <.0001		54
AMEn (kcal/kg)						42.0 ±3.85 <.0001	73.7 ±9.26 <.0001	41.1 ±2.52 <.0001		62
NE (kcal/kg)		0.815 <.0001	0.2			-12.8 3.82 0.003	14.8 0.86 0.046			101
NE (kcal/kg)				0.820 <.0001	0.21	-10 3.71 0.042	14.8 0.18 0.007			112

CHAPTER 6 – Implications

Implications

The adoption of the Net Energy (NE) system in broiler chicken nutrition marks a significant departure from traditional approaches, such as the metabolizable energy system corrected for nitrogen, for expressing feed energy values. The successful implementation of the NE system in the coming years hinges on various factors, including enhanced research to deepen our understanding of poultry energy metabolism under diverse conditions, standardization of methodologies for energy metabolism studies in poultry, and accurate determination of feed's NE values. These steps are crucial for improving data quality and minimizing discrepancies between different laboratories.

The NE system's implementation into practical poultry nutrition, as proposed in prior studies, offers numerous benefits, including improved feed formulation accuracy, production efficiency, environmental sustainability, and economic outcomes in the poultry sector. Key advantages include:

Precision in Nutrient Delivery: The NE system offers a more precise method for predicting feed energy values, which closely aligns with the energy requirements of broiler chickens. This accuracy improves feed utilization efficiency and accurately depicts feed intake when compared with metabolizable energy, as illustrated in Figure 1. Here, the NE intake suggests that this system could be a key factor influencing feed intake. It is crucial to note that this proposition is hypothetical, given that feed intake regulation is influenced by multiple factors. However, the NE system may better represent the premise that birds consume feed to meet their energy requirements as expressed on this basis.

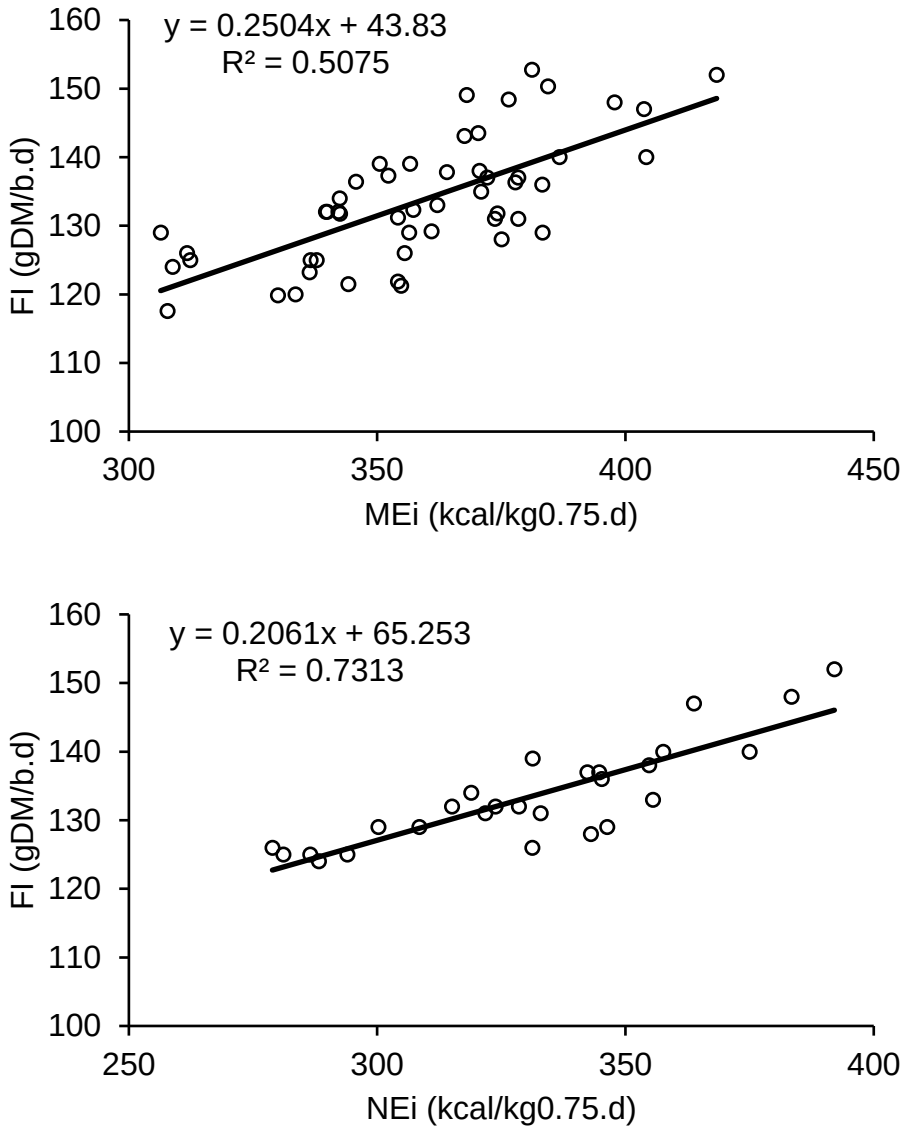

Figure 1. Relationship between metabolizavel energy intake (MEi) and net energy intake (NEi) versus the dry matter feed intake (FI) of broiler chickens (n=30)

Ingredient Flexibility and Cost-Effectiveness: A deeper understanding of the NE values of various ingredients allows for more informed decision-making in feed formulation, potentially broadening the ingredient spectrum without penalizing energy efficiency. This flexibility could lead to significant cost savings and reduced reliance on conventional, high-demand ingredients. Furthermore, accurately expressing "non-traditional" ingredients' NE values can offer economical feed alternatives, depending on market conditions, thereby

enhancing the profitability of poultry production. The implementation of the NE system can be facilitated by accurately predicting the energy values of various feedstuffs from different origins using their chemical composition. This tool could prove valuable for incorporating the NE system into practical feed formulation for poultry (Table 1 and Figure 2).

Table 1. Nutritional composition of feedstuffs as described on the Brazilian Tables for Poultry and Swine (Rostagno et al., 2017), and the net energy estimated based on the equations proposed on the Chapter 6.

Ingredients	Crude Protein, %	Ether Extract (EE)	AMEn, kcal/kg	NE[1], kcal/kg	NE[2], kcal/kg	NE[1]/AMEn
Millet, Grain	12.4	4.02	3189	2550	2577	0.80
Soybean Meal 46% CP	46.5	2.85	2396	1540	1935	0.64
DDGS	26.16	10.08	2851	2224	2303	0.78
Canola Meal	36.2	2.55	1743	1104	1408	0.63
Sunflower Meal	33.4	1.98	1795	1166	1450	0.65
Wheat, Grain	11.5	1.61	3039	2400	2455	0.79
Wheat Bran - Midds	15.1	3.4	1810	1383	1462	0.76
Barley, Grain	10.8	1.7	2701	2132	2182	0.79
Sorghum Grain	8.97	2.35	2956	2369	2388	0.80
Corn, Grain 7.86% CP	7.86	3.81	3364	2736	2718	0.81
Cottonseed Meal	37.8	2.65	1951	1260	1576	0.65

[1]Net energy value estimated based on the equation proposed on this work. NE= 0.82*AMEn-10.04*CP+14.8*EE.

[1]Net energy value of feedstuff based on the equation for broiler chickens proposed by Wu et al., (2019). NE=0.808*AMEn-0.017*CP+0.031*EE.

Based on the results presented, can be shown a similarities of the NE equation porpoused on the literature, demostrating the feasibility to predict the NE values based on the nutritional composition described on many tables of recommendations and guide for feed formulation (NRC, Feedna, CVB, Brazilian Tables for Poultry an Swine, etc.), and also on the analyzed values of ingredients and diets.

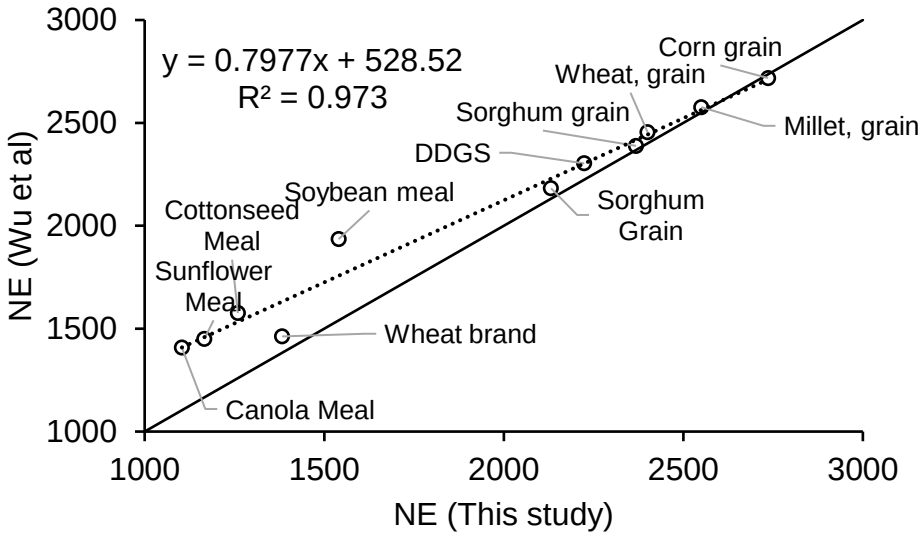

Figure 2. Estimated NE value of different feedstuffs used in poultry diets. The values were calculated based on the nutritional composition of Brazilian Tables (Rostagno et al., 2017).

Crude Protein Reduction and Nitrogen Management: Formulating feeds based on the NE system may facilitate the reduction of crude protein content, which in turn can decrease the diet's heat increment and enhance efficiency. This reduction has the added benefit of potentially lowering nitrogen emissions, aligning with environmental sustainability goals.

Conclusion

The transition to an NE system necessitates initial investments in research, training, and potentially new feed analysis and formulation technologies. However, the long-term benefits, such as cost reductions and improved product quality, will likely offset these initial expenses.

Overall, transitioning to an NE system in broiler chicken nutrition promises substantial improvements in feed formulation precision, production efficiency, environmental sustainability, and economic returns for the poultry industry. Achieving these benefits will require collective research, education, and infrastructure development efforts to embrace this advanced approach to energy evaluation in poultry diets.