

**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
CÂMPUS DE JABOTICABAL**

**EFEITO DO DESAFIO SANITÁRIO NA UTILIZAÇÃO DE
ENERGIA E NA PARTIÇÃO DA PRODUÇÃO DE CALOR EM
FRANGOS DE CORTE**

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**UNIVERSIDADE ESTADUAL PAULISTA – UNESP
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FRANGOS DE CORTE**

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para a obtenção do título de Doutora em Ciência Animal.

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Orientadora: Nilva Kazue Sakomura

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IMPACTO POTENCIAL DESTA PESQUISA

Esta pesquisa visa esclarecer os mecanismos do metabolismo energético em aves submetidas a desafios agudos e crônicos, com foco particular nos custos metabólicos associados às respostas imunológicas e fisiológicas, incluindo febre. Ao investigar como as aves ajustam sua retenção de energia, mobilizam o tecido adiposo e alteram as vias metabólicas para alimentar os processos imunológicos, este estudo contribuirá para uma compreensão mais profunda de como o equilíbrio energético é mantido sob estresse. Os insights obtidos com esta pesquisa podem revelar novas estratégias para mitigar os custos metabólicos das respostas imunológicas, otimizar a eficiência energética e melhorar a saúde geral e o desempenho das aves. Além disso, ao empregar a metabolômica, a pesquisa fornecerá uma compreensão detalhada em nível de sistemas dos processos bioquímicos subjacentes a essas respostas adaptativas, descobrindo biomarcadores específicos e vias metabólicas que podem ser direcionadas para melhorar o bem-estar das aves.

POTENTIAL IMPACT OF THIS RESEARCH

This research aims to clarify the mechanisms of energy metabolism in birds subjected to acute and chronic challenges, with a particular focus on the metabolic costs associated with immune and physiological responses, including fever. By investigating how birds adjust their energy retention, mobilize adipose tissue, and alter metabolic pathways to fuel immune processes, this study will contribute to a deeper understanding of how energy balance is maintained under stress. The insights gained from this research may reveal new strategies to mitigate the metabolic costs of immune responses, optimize energy efficiency, and improve overall health and performance in birds. Furthermore, by employing metabolomics, the research will provide a detailed, systems-level understanding of the biochemical processes underlying these adaptive responses, uncovering specific biomarkers and metabolic pathways that could be targeted to improve bird welfare.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: EFEITO DO DESAFIO SANITÁRIO NA UTILIZAÇÃO DE ENERGIA E NA PARTIÇÃO DA PRODUÇÃO DE CALOR EM FRANGOS DE CORTE

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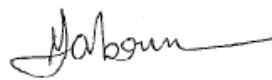
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Jaboticabal, 20 de setembro de 2024

DADOS CURRICULARES DO AUTOR

ROSIANE DE SOUZA CAMARGOS - nasceu em Carmo do Cajuru, MG no dia 17 de janeiro de 1997. Em março de 2016 ingressou no curso de Zootecnia no Instituto Federal de Minas Gerais - Campus Bambuí, MG, a qual foi monitora das disciplinas de avicultura e estatística experimental. No ano de 2017, desenvolveu sua primeira Iniciação Científica com bolsa do Instituto Federal de Minas Gerais – Campus Bambuí, intitulada “Restrição alimentar em coelhos visando maior rentabilidade” e em 2018 atuou como voluntária do projeto de extensão “Divulgando, Fomentando e Promovendo a Cunicultura: Canal DR CUNI, Boletim de Cunicultura e Curso de Cunicultura à Distância”, ambas sob orientação do Prof. Dr. Luiz Carlos Machado. No ano de 2019, desenvolveu sua segunda Iniciação Científica com bolsa da reitoria do Instituto Federal de Minas Gerais, intitulada “Suplementação de pigmentantes naturais na dieta para frangos caipira e sua influência na coloração da pele e da carne”, sob orientação do Prof. Dr. Adriano Geraldo. Em dezembro de 2020, graduou-se em Zootecnia. Em agosto de 2020, ingressou no curso de Mestrado em Ciência Animal no Laboratório de Ciências Avícolas da UNESP, Jaboticabal - SP, sob orientação da Prof^a. Dr^a. Nilva Kazue Sakomura, com bolsa financiada pela Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) de agosto de 2020 à julho de 2021, sob nº 33004102002P0, e em agosto de 2021 alterou-se o curso de Mestrado para o Doutorado Direto com bolsa financiada pela Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) até agosto de 2024, sob o nº de processo 2021/08142-5.

“Nunca perca de vista o seu ponto de partida”
(Santa Clara de Assis)

Dedico,

este trabalho primeiramente a Deus por nortear a minha vida. A meus pais José Nilton e Luciana por acreditarem e apoiarem meu sonho. Aos meus irmãos Rodrigo e Renata pelo exemplo e incentivo. E as minhas cachorrinhas Filó e Lola, que sempre me trouxeram conforto e me ensinam o verdadeiro sentido da palavra amor.

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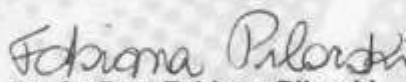
CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Efeito do desafio sanitário no particionamento da produção de calor e na utilização de energia em frangos de corte"**, protocolo nº 5399/20, 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	23/05/2021 a 30/06/2021
Espécie / Linhagem	<i>Gallus gallus domesticus</i> / Cobb 500
Nº de animais	100
Peso / Idade	40 g / 1 dia
Sexo	Machos
Origem	Incubatório Pluma, Descalvado - SP

Jaboticabal, 10 de dezembro de 2020.


Profa. Dra. Fabiana Pilarski
Coordenadora – CEUA

EFEITO DO DESAFIO SANITÁRIO NA UTILIZAÇÃO DE ENERGIA E NA PARTIÇÃO DA PRODUÇÃO DE CALOR EM FRANGOS DE CORTE

RESUMO – O estímulo do sistema imunológico do hospedeiro resulta na redução do crescimento, devido à utilização de fontes endógenas para garantir a sobrevivência, prejudicando a deposição tecidual e resultando em menor ganho de massa corporal. Sob desafios de saúde, as fontes de energia são utilizadas para garantir funções essenciais e apoiar o sistema imunológico. Porém, as alterações no metabolismo energético quando as aves são desafiadas pelo estresse imunológico ainda não são totalmente compreendidas, sendo essencial entender o uso da energia diante de um desafio sanitário e as alterações no metabolismo. O objetivo da presente tese foi avaliar as adaptações fisiológicas e metabólicas, e seu impacto na utilização de energia de frangos de corte submetidos a diferentes modelos de desafios sanitários. Os modelos de desafio utilizados foram Ensaio 1: indução de enterite necrótica (*Eimeria maxima* e *Clostridium perfringens*) e Ensaio 2: desafio por lipopolissacarídeo de *Escherichia coli*. No primeiro ensaio foram utilizados 96 frangos de corte machos Cobb-500 criados em gaiolas até completarem 12 dias de idade. Aos 13 d, as aves foram distribuídas aleatoriamente nos tratamentos experimentais: AL (consumo de ração *ad libitum* sem desafio), C (aves desafiadas) e PF (aves não desafiadas com alimentação pareada em referência ao grupo C). As aves foram alocadas em câmaras respirométricas para habituação por um período de um dia. O protocolo de desafio consistiu na inoculação de *E. maxima* aos 14 d e *C. perfringens* aos 18, 19 e 20 d, ambos procedimentos via gavagem oral. De 14 a 22 d foi mensurado o consumo de ração, peso vivo, produção de excretas, consumo de O₂ e a produção de CO₂. No ensaio 2 foram utilizados 21 frangos de corte machos Cobb-500, criados em gaiolas até 18 dias de idade. Aos 19 d, as aves foram distribuídas em três tratamentos: Controle não desafiado (CON), aves desafiadas com 10 µg LPS/kg PV (10LPS) e 100 µg LPS/kg PV (100LPS) da cepa LPS de *E. coli*. Aos 21 d, o LPS foi administrado por via intramuscular no peito. As variáveis de trocas gasosas e temperatura corporal interna foram registradas continuamente durante 23 horas em condições de jejum. Aos 22 d, foi realizada a coleta de sangue e fígado para análise do perfil de metabólitos. No ensaio 1, os resultados demonstram que o tratamento AL apresentou maior consumo de ração e peso corporal que os tratamentos PF e C (P<0,05). O desafio patogênico influenciou notavelmente na utilização de energia, com uma redução significativa no consumo de energia metabolizável (P<0,05) e se observou uma mobilização da energia de fontes endógenas do tecido lipídico (P<0,05). A diminuição da eficiência energética residual foi observada como um efeito específico do desafio (aproximadamente 20%) e à anorexia induzida pela doença (aproximadamente 10%). No ensaio 2, observou-se que o tratamento 100LPS apresentou uma menor produção de calor (P<0,05) comparado ao grupo CON, e semelhante ao 10LPS (P>0,05). O quociente respiratório e a temperatura corporal foram maiores no 10LPS (P<0,05). Esta variação no metabolismo resultou de uma diminuição da abundância de metabólitos (aminoácidos, vitaminas, ácidos graxos e outros) refletindo seu uso em aves desafiadas (10LPS e 100LPS), em relação ao tratamento CON. Os resultados desta pesquisa evidenciam que as aves desafiadas reduzem o consumo voluntário de ração, afetando o crescimento. Sendo este o resultado da alteração no metabolismo

energético das aves para garantir a resposta imunológica. Os desafios patogênicos ocasionam redução das taxas de retenção de energia e mobilização do tecido adiposo como fonte de energia para resposta imunológica em desafios prolongados. Por outro lado, as aves desenvolvem processos febris sob desafios prolongados e sistêmicos. Estas alterações na utilização de energia e na resposta fisiológica é evidenciada pela alteração no perfil de metabólitos. Compreender estas respostas aos desafios patogênicos pode ajudar a desenvolver estratégias eficazes para otimizar o crescimento das aves.

Palavras-chave: Desafio Patogênico, Gasto energético, Metabolismo Energético, Metabolômica, Produção de Calor, Temperatura corporal

EFFECT OF THE HEALTH CHALLENGE ON ENERGY UTILIZATION AND HEAT PRODUCTION PARTITIONING IN BROILER CHICKENS

ABSTRACT – The stimulation of the host's immune system results in reduced growth due to the utilization of endogenous sources to ensure survival, impairing tissue deposition and leading to lower body mass gain. Under health challenges, energy sources are used to ensure essential functions and support the immune system. However, the changes in energy metabolism when birds are challenged by immune stress are still not fully understood, making it essential to understand energy use in the face of a health challenge and the alterations in metabolism. The objective of this thesis was to evaluate the physiological and metabolic adaptations and their impact on energy utilization in broilers subjected to different health challenge models. The challenge models used were: Trial 1: induction of necrotic enteritis (*Eimeria maxima* and *Clostridium perfringens*) and Trial 2: challenge by *Escherichia coli* lipopolysaccharide. In the first trial, 96 male Cobb-500 broilers were raised in cages until they were 12 days old. At 13 days, the birds were randomly distributed into experimental treatments: AL (*ad libitum* feed consumption without challenge), C (challenged birds), and PF (unchallenged birds with paired feeding reference to group C). The birds were placed in respirometry chambers for a day of habituation. The challenge protocol consisted of inoculation of *E. maxima* at 14 days and *C. perfringens* at 18, 19, and 20 days, both procedures via oral gavage. From 14 to 22 days, feed intake, body weight, excreta production, O₂ consumption, and CO₂ production were measured. In trial 2, 21 male Cobb-500 broiler chicks were raised in cages until 18 days old. At 19 days, the birds were distributed into three treatments: non-challenged control (CON), challenged broilers with 10 µg LPS/kg BW (10LPS) and 100 µg LPS/kg BW (100LPS) of the *E. coli* LPS strain. At 21 days, LPS was administered intramuscularly in the breast. Gas exchange variables and internal body temperature were recorded continuously for 23 hours under fasting conditions. At 22 days, blood and liver samples were collected for metabolite profile analysis in each tissue. In trial 1, the results showed that the AL treatment had higher feed intake and body weight than the PF and C treatments ($P<0.05$). The pathogenic challenge notably influenced energy utilization, with a significant reduction in metabolizable energy intake ($P<0.05$) and mobilization of energy from endogenous lipid tissue sources ($P<0.05$). The decrease in residual energy efficiency was observed as a specific effect of the challenge (approximately 20%) and disease-induced anorexia (approximately 10%). In trial 2, it was observed that the 100LPS treatment had lower heat production ($P=0.05$) compared to the CON group and similar to the 10LPS ($P>0.05$). The respiratory quotient and body temperature were higher in 10LPS ($P<0.05$). This variation in metabolism resulted from a decrease in the abundance of metabolites (amino acids, vitamins, fatty acids, and others) in challenged birds (10LPS and 100LPS) compared to the CON treatment. The results of this research show that challenged birds reduce voluntary feed intake, affecting growth. This is a result of the altered energy metabolism of birds to ensure the immune response. Pathogenic challenges lead to reduced energy retention rates and mobilization of adipose tissue as an energy source for the immune response in prolonged challenges. On the other hand, birds develop febrile processes under prolonged and systemic challenges. These changes in energy utilization and physiological response are evidenced by the

altered metabolite profile. Understanding these responses to health challenges can help develop effective strategies to optimize bird growth.

Keywords: Pathogenic Challenge, Energy Expenditure, Energy Metabolism, Metabolomics; Heat Production, Body Temperature

CAPÍTULO 1 – Considerações Gerais

Introdução

O crescimento acelerado de frangos de corte modernos está atrelado ao seu alto consumo de ração e elevada eficiência de utilização dos nutrientes. No entanto, distúrbios no estado de saúde das aves ocasionam uma redução do consumo voluntário de ração e diminuição na eficiência de utilização de nutrientes e energia, pois uma fração da energia é destinada para atender as demandas da resposta imune, sendo um dos principais efeitos deletérios durante o desenvolvimento da doença (Arai, 2014; M'Sadeq et al., 2016).

A ativação da resposta imune é o principal mecanismo de defesa que garante a sobrevivência do hospedeiro (Lochmiller e Deerenberg, 2000), e pode ser classificada como um mecanismo inato e adaptativo, atuando em curto e longo prazo, respectivamente (Humphrey e Klasing, 2004). A resposta imune protege o organismo contra patógenos, objetivando manter o equilíbrio homeostático. Esta ativação mantém a homeostase tecidual para garantir um balanço entre a síntese e degradação em proporções que não prejudiquem o organismo. Como as células que compõem o sistema imune carecem de estoques contínuos de nutrientes, essas respostas efectoras só podem ser sustentadas por meio de um aumento drástico na utilização e aproveitamento de glicose, aminoácidos e ácidos graxos de seu microambiente (Ganeshan e Chawla, 2014). Essa ativação requer energia tanto advinda do alimento, e principalmente das reservas endógenas, acarretando distúrbios na utilização de nutrientes (Kogut, 2009), consequentemente impacta no desempenho do animal.

A utilização e destino metabólico dos nutrientes para resposta imune auxilia principalmente a dois propósitos importantes. Primeiro, fornecem os substratos para a síntese de Adenosina Trifosfato (ATP) e sustentam os diversos mecanismos de ação das células imunes ativadas. Em segundo lugar, fornecem os blocos de construção (monômeros) para a síntese de macromoléculas (oligoproteínas e fosfolipídios), que são necessárias para a proliferação e ação de células do sistema imune (Ganeshan e Chawla, 2014). Nesse sentido, o suprimento e uso dos nutrientes pela unidade celular, e consequentemente pelo organismo, tornam-se diferentes sob condições de desafio comparado a uma

condição sadia. Além disso, o uso dos nutrientes pode variar dependendo do grau da infecção, tipo de agente patogênico, entre outros, que principalmente se reflete na ação e tipo da endotoxina (Mohammad e Thiemermann, 2021).

Nesse sentido, sob condições de estresse imunológico, o anabolismo de tecido proteico e lipídico será reduzido, enquanto que haverá um aumento na taxa catabólica destes tecidos, com intuito de alcançar a exigência de nutrientes para sintetizar moléculas efetoras imunológicas. Adicionalmente, há uma mudança na partição de nutrientes dietéticos direcionado para as respostas metabólicas, que dão suporte ao sistema imunológico (Johnson, 1997). A ativação da resposta imunológica, ocasiona alterações, devido à resposta febril e reações inflamatórias, que consomem recursos consideráveis, esgotando estoques de carboidratos e catabolizando proteínas (Iseri e Klasing, 2013).

Esta alteração no equilíbrio térmico interno do indivíduo, faz parte do componente termorregulador da resposta imune a um patógeno, e pode ser um custo-chave no orçamento energético de uma ave, quando a resposta induzida é a febre (Marais et al., 2011). A febre, definida como hipertermia regulada, é parte de uma reação fisiológica intrincada a patógenos invasores, conhecida como resposta de fase aguda. Durante a febre, o sistema termorregulador é ajustado de uma maneira que favorece a retenção de calor e a produção de calor, enquanto os mecanismos de perda de calor são reduzidos. Para frangos de corte, nessas condições, o metabolismo energético terá um impacto direto na ingestão de ração, composição da carcaça e eficiência alimentar, sendo crucial entender o que ocorre por trás desse metabolismo, relacionado as respostas metabólicas e; também as alterações das rotas metabólicas dos nutrientes e seu perfil de metabólitos.

A metabolômica é uma ferramenta que desempenha um papel crucial na compreensão das respostas bioquímicas e fisiológicas de aves submetidas a desafios patogênicos, esta abordagem permite a análise abrangente dos metabólitos dentro do organismo, esclarecendo as mudanças nas vias metabólicas (Wu et al., 2022). Quando as aves enfrentam um desafio sanitário, o seu metabolismo sofre alterações significativas para satisfazer as crescentes exigências energéticas e para manter a homeostase interna. A metabolômica ajuda a identificar as mudanças nos principais processos metabólicos, como o metabolismo da glicose e dos lipídios, que são as principais fontes para a

produção de ATP, a moeda energética das células (Weckmann et al., 2018). Além disso, revela a ativação de vias específicas envolvidas na resposta imunológica e na adaptação ao estresse, fornecendo informações sobre como as aves gerenciam os recursos energéticos durante a doença.

Em geral, o estímulo no sistema imunológico de um hospedeiro resulta em redução no crescimento, devido ao uso de fontes endógenas e alocação dos recursos, objetivando a sobrevivência e prejudicando a deposição dos tecidos, resultando em menor ganho de massa corporal. Sob desafios de saúde, as fontes de energia são utilizadas para garantir as funções essenciais e o suporte do sistema imunológico (Van Eerden, 2007). No entanto, as mudanças do metabolismo energético quando as aves são desafiadas pelo estresse imunológico ainda não é totalmente compreendida, sendo essencial conhecer a utilização de energia diante de um desafio sanitário, onde o animal e seu metabolismo são modulados para readequação e atendimento das exigências nutricionais nesta condição.

O objetivo da tese foi avaliar as alterações metabólicas e fisiológicas de aves submetidas a diferentes modelos de desafio, e seu impacto na utilização de energia e as adaptações no perfil de metabólitos.

Revisão de Literatura

Metabolismo Energético

O metabolismo é um conjunto de processos metabólicos de anabolismo e catabolismo que acontecem no interior da célula. O metabolismo energético constitui um conjunto de processos (vias metabólicas) que produzem energia em forma de ATP, a partir da oxidação dos nutrientes (Nelson e Cox, 2014). A geração de ATP envolve o catabolismo de macronutrientes, cada um começando de uma via metabólica diferente, mas finalmente sendo transportado para as células para produção de ATP. Fatores que podem afetar negativamente a produção de ATP, incluem, disponibilidade reduzida de glicose intracelular por meio de alterações no metabolismo de macronutrientes ou redução da captação de glicose pelas células, funcionamento reduzido das mitocôndrias para produção de energia aeróbica e subsequente aumento da dependência de glicólise aeróbica menos eficiente (Lacourt et al., 2018).

O catabolismo, refere-se à hidrólise de moléculas complexas (carboidratos, lipídios e proteínas), em seus respectivos monômeros (monossacarídeos, ácidos graxos e aminoácidos). O catabolismo é um processo exotérmico que libera energia, tendo como coprodutos o ATP e cofatores enzimáticos que participam na cadeia transportadora de elétrons (NADH, NADPH, FADH₂ e GTP). Já o anabolismo, requer energia para as reações de síntese, onde precursores simples são utilizados para formar moléculas maiores e complexas (lipídios, ácidos nucleicos, polissacarídeos e proteínas) (Nelson e Cox, 2014).

A principal fonte de energia nas aves provém do alimento, onde os nutrientes são digeridos, absorvidos e utilizados. Já a energia contida nas macromoléculas é liberada por meio de processos oxidativos e é utilizada em forma de ATP, para ser empregada como fonte de energia nos processos metabólicos (Nelson e Cox, 2014). Portanto, a energia não é considerada um nutriente, mas sim uma propriedade da oxidação dos nutrientes durante os processos metabólicos.

A energia dos alimentos pode ser expressa em diferentes bases, dependendo dos processos nos quais são avaliadas. A primeira está relacionada aos processos digestivos, que consiste na ação enzimática nos macronutrientes que acontece no trato gastrointestinal, e a sua subsequente absorção de

moléculas mais simples nos enterócitos. A segunda, ocorre no citoplasma celular, onde a maioria dos açúcares, ácidos graxos, glicerol e aminoácidos são convertidos em acetil-CoA, para serem utilizados no ciclo de Krebs na matriz mitocondrial, gerando uma pequena quantidade de ATP. Já a terceira fase, ocorre na membrana mitocondrial, onde o ATP é sintetizado a partir dos cofatores enzimáticos reduzidos por meio do ciclo da fosforilação oxidativa (Nelson e Cox, 2014).

Entretanto, os sistemas de avaliação de energia utilizados nos alimentos, segue o seu particionamento em termos de energia bruta, digestível, metabolizável e líquida. Dado que, a avaliação do metabolismo energético em termos de ATP apresenta limitações, decorrente da sua alta dinâmica de fosforilação, e a sua dificuldade de representar o valor energético do alimento.

Partição da Energia

A energia total contida nos alimentos é determinada pela combustão completa de todos os componentes orgânicos presentes, sendo metodologicamente determinada em uma bomba calorimétrica. Este potencial energético é denominado energia bruta (EB), e depende de sua composição química em termos dos macronutrientes (Figura 1). A energia digestível (ED), corresponde à energia dos alimentos que foi absorvida após o processo de digestão, calculada pela diferença entre a EB do alimento ingerido e a EB presente nas fezes. A energia metabolizável aparente corrigida pelo balanço de nitrogênio (EMAn), é a ED menos a energia perdida na urina e nos gases, sendo a principal expressão de energia em aves, já que leva em consideração a fração excretada como urina e fezes em conjunto. Adicionalmente, devido à baixa quantidade de energia perdida na forma de gases em aves, esse valor geralmente é desconsiderado nos cálculos (Sakomura e Rostagno, 2016). O sistema de EMAn é atualmente o sistema energético mais empregado na formulação de rações comercial de aves, tanto para avaliar o valor energético dos alimentos quanto para descrever as necessidades energéticas dos animais.

Durante o processo de digestão, absorção e metabolismo do alimento, há uma fração de energia perdida como incremento calórico (IC). O IC é calculado pela diferença entre a produção de calor total (PCT) e a produção de calor em jejum (PCJ). O IC está estritamente relacionado ao consumo e às características

da dieta. A energia líquida (EL) é obtida da EMAn subtraindo a energia perdida como IC, representando a quantidade de energia disponível para manutenção, crescimento e produção (ex. ovos) (Sakomura e Rostagno, 2016). Portanto, a EL pode ser subdividida em EL de manutenção (ELm) e EL de produção (ELp). A ELm é a energia utilizada para manter as funções vitais dos animais (Noblet, 2007) e geralmente é estimada através da PCJ (Noblet et al., 2015). A ELp, descrita a partir da energia retida (ER) como tecido, representa a fração de energia ingerida que é utilizada para síntese de tecidos e formação dos produtos (NRC, 1994). A ER no corpo, predominantemente como proteína e gordura, pode ser medida através da técnica do abate comparativo (Sakomura e Rostagno, 2016) ou pelo balanço de energia (Koh e Macleod, 1999).

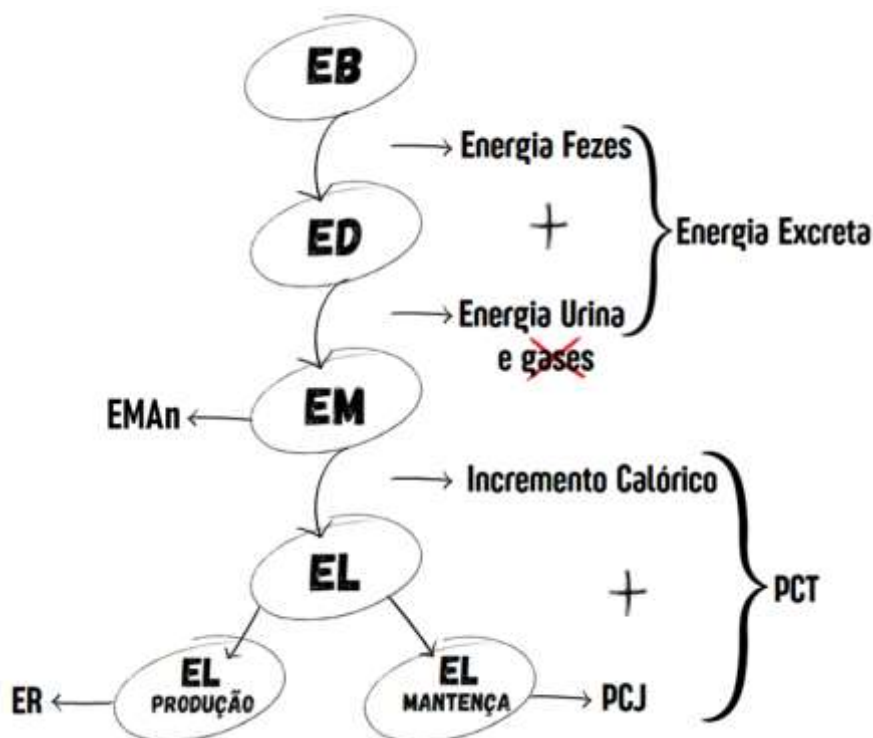


Figura 1. Partição da energia em aves.

Sistemas de energia

O sistema de EMA apresenta algumas imprecisões, apesar de ser amplamente adotado na avicultura. Este sistema não considera as perdas de energia decorrentes dos processos digestivos e metabólicos, considerados dentro do IC, que está atrelado às características da dieta, sendo considerado a fração inerente do alimento. Ao considerar as perdas de energia por IC no sistema de EMA, surge o sistema de EL, que representa o sistema que se

aproxima ao teor energético dos alimentos complementar às necessidades dos animais, pois considera as variações na utilização da EMA entre os nutrientes e o IC da dieta (Van Milgen et al., 1997). A determinação metodológica para quantificar a produção de calor (PC), é realizada principalmente pelo método de calorimetria (McLean and Tobin, 1987). Adicionalmente, este particionamento da produção de calor em IC e PCJ, por questões práticas, considera apenas aves mantidas sob condições de conforto térmico, e sem nenhum fator estressor que interfira este fracionamento clássico. No entanto, diversas condições estressoras podem influenciar neste particionamento, como por exemplo o desafio sanitário, que demanda uma quantidade de energia para ativação da resposta imune, assim como, modifica o comportamento do indivíduo, alterando principalmente às necessidades energéticas em comparação a uma ave sadia. Nesse sentido, o sistema de EL, a partir da avaliação acurada do uso de energia, pode representar melhor estas condições nas exigências de energia das aves em comparação com o sistema de EMA.

Calorimetria

A calorimetria é uma metodologia para a avaliação da PC através de abordagens físicas (calorimetria direta - CD) ou pela análise quantitativa de subprodutos químicos do metabolismo (calorimetria indireta - CI) (McLean e Tobin, 1987). A diferença entre as duas, é que na CD, a energia térmica gerada pelo organismo é medida pela variação da temperatura entre dois sistemas (animal e ambiente), avaliando a taxa de perda de calor de um organismo, por meio de funções de transferência de energia, de um gradiente de alta para outro de baixa. Já a CI, avalia a PC com base na mensuração quantitativa dos subprodutos e reações oxidativas do metabolismo.

A CD apresenta desafios em sua aplicação, uma vez que requer monitoramento contínuo dos equipamentos e controle preciso da temperatura na câmara isolada, o que torna a análise complexa e detalhada. Além disso, esse método presume que nenhum calor é retido no corpo do organismo durante a medição, e requer de funções matemáticas complexas baseadas nessa pressuposição. A CI mensura a quantidade molar ou volumétrica de oxigênio (O₂) consumido e dióxido de carbono (CO₂) produzido, durante os processos oxidativos de diversos substratos, possibilitando o cálculo da PC com base nos

princípios do balanço estequiométrico. Para calcular a PC em estudos utilizando CI, tem sido empregada a equação proposta por Brouwer (1965), que fundamenta na medição do volume de O₂ ($\dot{V}O_2$) e volume de CO₂ ($\dot{V}CO_2$), liberados na oxidação dos nutrientes, como gordura, carboidrato e proteína.

$$PC \text{ (kcal/kg}^{0.75}\text{/d)} = 3,86 \times \dot{V}O_2 + 1,20 \times \dot{V}CO_2$$

Os distintos substratos apresentam variações no consumo de O₂ e na produção de CO₂. Com a razão da troca respiratória ou quociente respiratório (QR) ($\dot{V}CO_2/\dot{V}O_2$), é possível avaliar a oxidação dos nutrientes envolvidos no metabolismo animal, de modo que para carboidratos, proteínas e gorduras apresentam valores em torno de 1,0; 0,8 e 0,7, respectivamente (Sakomura e Rostagno, 2016). Assim, se o animal estiver em estado de jejum este quociente estará por volta de 0,7 indicando o catabolismo de gordura em uma maior taxa, enquanto no estado alimentado, o QR estará próximo de 1,0, devido à oxidação de carboidratos provenientes da dieta, como fonte principal de energia (Gerrits et al., 2015).

➔ **Tipos de Sistemas de Calorimetria Indireta**

Existem diversos sistemas de CI e as suas adaptações para cada condição na pesquisa. Uma forma de classificação pode ser dependendo do circuito e fluxo de gases conduzidos para o sistema, sendo estes categorizados como sistemas de circuito aberto ou fechado. No sistema de CI de circuito aberto, o animal inspira ar do ambiente, que passa através da câmara de respirometria. O ar de entrada e saída da câmara são analisados quanto à sua composição de O₂ e CO₂. A diferença entre a concentração de gás de entrada e saída, é utilizada para calcular o consumo volumétrico de O₂ e a produção de CO₂, considerando o fluxo dos gases (Sakomura e Rostagno, 2016).

Nos sistemas de circuito fechado, uma concentração conhecida dos gases (principalmente oxigênio) é fornecido, e reciclado dentro do sistema, de forma que, o ar inspirado pelo animal provém de uma única fonte variando a sua concentração de O₂ ao longo do tempo, enquanto o CO₂ expirado é absorvido por uma solução de KOH. O consumo de O₂ é calculado pela diferença de peso da subamostra de O₂ inicial e final, enquanto que a produção de CO₂ pode ser recuperado da solução de KOH por técnicas de precipitação (Lighton, 2008).

Em ambos os sistemas, o ar analisado ou CO₂ reciclado, é desidratado utilizando sílica ou substâncias de alto poder higroscópico, para absorver a

umidade proveniente da respiração, sendo considerado a fração livre de vapor de água nas análises.

Particionamento da produção de calor

A PCT de um animal, reflete sua taxa metabólica e pode ser influenciada por vários fatores, como temperatura ambiente, estado fisiológico, comportamento, tipo de alimentação, entre outros (McLean and Tobin, 1987). A PCT é tradicionalmente particionada em IC e a PCJ. A energia ingerida pelo animal, após desconsiderar a fração perdida nas excretas, é destinada principalmente para o suporte das funções vitais, considerado a PCJ, sendo posteriormente direcionada para síntese dos tecidos e aumento da massa corporal, e o excesso de energia é perdido em forma de IC. Além do particionamento tradicional da PCT, um terceiro componente associado à atividade física é considerado em condições normais de criação (Zuidhof, 2019).

Este particionamento se baseia no fracionamento para isolar o efeito dos fatores externos ao animal, e poder avaliá-los de forma separada. Uma forma de particionamento é descrita por van Milgen (1997), onde a PCJ representa o metabolismo basal do indivíduo e considera um valor constante expresso por unidade de peso metabólico. Por outro lado, o IC, influenciado diretamente pelas características físico-químicas da dieta, pode ser fracionado como efeito térmico do alimento a curto e longo prazo, apresentando a sua principal diferença decorrente da ingestão e digestão do alimento de resposta a curto prazo, enquanto a absorção, transporte e uso metabólico, caracteriza uma resposta a longo prazo. Adicionalmente, a PC pelo efeito da atividade física, representa a variação da taxa metabólica decorrente das atividades locomotoras do indivíduo.

Nesse sentido, os protocolos para a determinação da PCJ consideram a mensuração da PC de um animal durante o estado metabólico de jejum, envolvendo o catabolismo das reservas energéticas, principalmente dos depósitos de gordura corporal, para manter os processos metabólicos básicos e, portanto, a sobrevivência do animal (Fernández et al., 2012). A determinação da taxa metabólica basal, medida pela PCJ, requer condições específicas durante a sua avaliação, incluindo um mínimo de atividade física, estado pós-absortivo, termoneutralidade e inatividade circadiana (Bennett e Harvey, 1987).

Outros componentes no particionamento energético podem ser considerados, dependendo das condições ambientais, como a variação da temperatura, sendo necessário quantidades adicionais de demanda energética para condições abaixo da termoneutralidade (termogênese) e para condições acima da termoneutralidade (termólise) (Abreu-Vieira et al., 2015). Outros fatores indutores de estresse também podem influenciar na variação da PC, como por exemplo o efeito da infecção sobre a taxa metabólica (Merchant et al., 2024).

O sistema imunológico é frequentemente considerado em termos de suas respostas a patógenos, como proliferação de leucócitos, produção de anticorpos ou aumento rápido na síntese de proteínas de fase aguda. Esses tipos de defesas imunológicas são conhecidos por serem energeticamente dispendiosos (Shattuck-Heidorn et al., 2017). A infecção afeta a disponibilidade de energia celular por meio de seus efeitos no metabolismo, ao mesmo tempo, afeta o gasto de energia, tanto por meio do aumento da demanda de energia pelo sistema imunológico, quanto por meio de mudanças no gasto de energia.

Custo Metabólico e Resposta Imune

A eficiência da utilização de energia pelos animais durante o crescimento é influenciada por uma série de fatores, incluindo composição da dieta, sexo e linhagem do animal, além do estado fisiológico. A eficiência energética líquida, representa a proporção da energia total ingerida que não é excretada nas fezes, urina ou como termogênese induzida pela dieta e, portanto, está disponível para as necessidades energéticas essenciais, para atividades físicas e, para síntese de tecidos e crescimento (Donato, 1987).

A taxa metabólica basal é definida como a taxa de metabolismo energético de um organismo que não está em fase de crescimento, enquanto está em repouso, sob condições pós-absortivas em termoneutralidade, representando o gasto energético mínimo para manter as células e tecidos ativos para atividade de alta intensidade, e a capacidade metabólica de disponibilizar energia das fontes endógenas para as atividades essenciais (Bligh e Johnson, 1973). No entanto, essas características se aplicam a condições em que o indivíduo está sadio e em termoneutralidade, sem qualquer alteração que ocasionem distúrbios no metabolismo.

Em condições estressoras causada por agentes patogênicos, a alocação de energia seguirá estratégias que assegurem a sobrevivência do organismo. Tais respostas e reações, demandam um alto custo energético para o animal, aumentando o gasto energético total, comparado a um animal sadio. Torna-se necessário obter energia imediata por meio do catabolismo dos tecidos de reserva, aumentando a PC da ave e reduzindo significativamente a retenção energética nos tecidos (Adedokun e Olojede, 2019).

É amplamente reconhecido que o estado nutricional ou energético de um animal, pode ter um impacto significativo em sua resposta a patógenos e parasitas (Klasing, 1998). O grau de nutrição pode, portanto, influenciar a 'resiliência' e a 'resistência' do hospedeiro à infecção. Resiliência pode ser vista como a habilidade do hospedeiro de manter um nível aceitável de produtividade, diante de um desafio parasitário, enquanto a resistência é uma medida da capacidade do hospedeiro de limitar o estabelecimento, taxa de crescimento, fecundidade e/ou persistência de uma população de parasitas (Albers et al., 1987).

Diversos estudos mencionam que um desafio de patógeno pode levar a um aumento nas demandas energéticas devido à produção de proteínas imunes (Demas et al., 1997), transporte e excreção de nitrogênio (Sykes e Coop, 1977) e principalmente à expressão de febre (Baracos et al., 1987), o que requer maior energia em fases agudas (Figura 2).

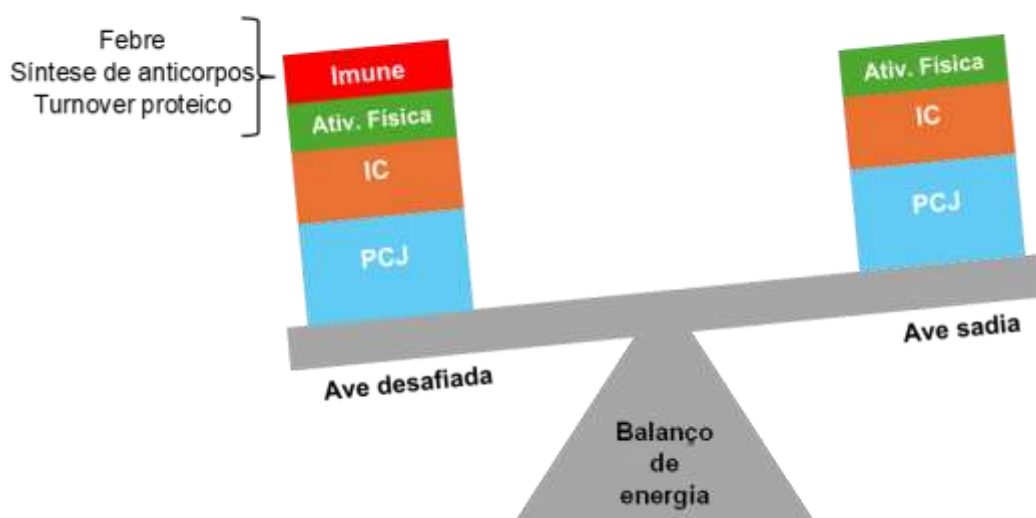


Figura 2. Balanço energético de aves sadias e desafiadas.

A proporção entre a perda de tecido adiposo e proteína (especialmente proteína muscular), depende do estado inflamatório do corpo, que diminui significativamente a capacidade de adaptação a um balanço energético negativo. A perda de proteína corporal é muito mais rápida durante a doença inflamatória, do que durante a fome (Singer et al., 2019), como resultado de uma acelerada taxa de turnover proteico. Isso ocorre porque a inflamação sistêmica leva a um aumento na demanda por aminoácidos para fins de resposta inflamatória, o que leva a uma perda gradual de proteínas do músculo esquelético.

Sistema imunológico

A defesa imunológica é um mecanismo vital para proteger o organismo contra infecções e lesões, porém requer um alto custo metabólico, uma vez que sua ativação está condicionada pela presença de fatores estressores (Zuk e Stoehr, 2002). Além dos danos e da imunopatologia, a ativação da resposta imune implica em uma mudança metabólica para o hospedeiro (Odegard e Chawla, 2013).

As fases da resposta inflamatória direcionadas para os patógenos, a migração celular e a proliferação celular, demandam uma grande quantidade de energia, exigindo a redistribuição de nutrientes para sustentar a ativação imunológica (O'Neill e Pearce, 2016). Por outro lado, o comportamento doente é acompanhado pela anorexia, reduzindo a ingestão de nutrientes provenientes dos alimentos e tornando necessária a mobilização de recursos para a sobrevivência do indivíduo (Wang et al., 2016).

A resposta do hospedeiro a entrada de algum patógeno é influenciada por vários fatores como, a carga microbiana patogênica, natureza da ameaça (viral, bacteriana, fúngica), duração da infecção, localização (extracelular, intracelular), virulência e *status* de replicação do patógeno. As fases da resposta imune, incluem os mecanismos não específicos que representam a primeira barreira de resistência e combate ao patógeno (principalmente por fagocitose), em seguida as células responsáveis pelo reconhecimento da ameaça (células B), seguida da produção de substâncias (síntese de proteínas chamadas de anticorpos),

finalizando na tolerância para o resguardo do hospedeiro em infecções futuras pelo mesmo patógeno (Riera Romo et al., 2016).

A resposta generalizada a uma infecção é para o corpo redistribuir os recursos nutricionais e energéticos longe dos processos anabólicos, e de manutenção para os processos metabólicos vitais, impulsionando a imunidade e resistência a doenças (Barthelemy et al., 2023). Essa resposta começa com o sistema fagocítico mononuclear e ocorre a liberação de uma série de mensageiros secundários. Lipólise acelerada, proteólise e suprimento de glicólise são necessários para montar o sistema imunológico inicial, em resposta à infecção, resultando em perda substancial de peso corporal, se as infecções forem prolongadas e graves (Lochmiller e Deerenberg, 2000).

Alguns desses custos podem estar ligados a mecanismos pró-inflamatórios, que são valiosos para destruir patógenos, mas também podem causar danos ao hospedeiro. Uma consequência de combater uma infecção é uma redução no consumo de ração, uma resposta adaptativa-comportamental do organismo, para reduzir o risco de novas infecções. Essa anorexia induzida pela sepse é ocasionada no momento em que o corpo precisa de um influxo de nutrientes para suportar as demandas crescentes de construir uma resposta imune (Scrimshaw, 1977). Consequentemente, processos catabólicos devem ser ativados, para suportar as necessidades adicionais de combustível das células imunes e síntese de proteínas.

Custos metabólicos para manter um sistema imunológico ativo são extremamente complexos de serem mensurados. A restrição de energia na dieta, se prolongada, pode levar à supressão do sistema imunológico e aumentam os riscos de infecção, especialmente com patógenos oportunistas (Klurfeld, 2013). Ao contrário da fome, onde o hospedeiro eventualmente se ajusta fisiologicamente para a redução do aporte de nutrientes por diminuição do metabolismo, a infecção leva a um hiper estado metabólico para apoiar a regulação positiva do sistema imunológico. Para alimentar essa regulação, as células imunológicas requerem glicose e glutamina em níveis elevados (Crouser e Dorinsky, 1996), o que leva ao rápido colapso das fontes de carboidratos circulantes, reservas de proteína e lipídio do corpo (Michie, 1996).

Cerca de 20% da proteína mobilizada do músculo esquelético é usada para satisfazer essas demandas energéticas (Duke et al., 1970), o restante é

alimentado por carboidratos e lipídios. Borel et al. (1998) estimou que cerca de 50% da elevação da taxa metabólica durante uma infecção, pode ser atribuída ao elevado custo energético da síntese proteica. Durante o período de recuperação, um animal pode ser confrontado com custos nutricionais associados à reposição das reservas de tecidos corporais, que foram esgotadas durante o ciclo catabólico. O custo energético de sintetizar novas proteínas para substituir aquelas perdidas no músculo esquelético devem ser adicionadas ao custo total da resposta imune. Sendo assim, os hospedeiros infectados podem se proteger usando diferentes estratégias de defesa, sendo elas inata e adaptativa (Soares et al., 2017).

Os mecanismos de reconhecimento do sistema imune inato têm um custo energético reduzido e constante e, é considerado como uma fração do metabolismo basal (Råberg et al., 2002), que envolve principalmente a síntese de proteínas de reconhecimento. Enquanto no início de um processo inflamatório agudo, que consiste em uma série de reações induzidas pelo sistema imune adaptativo em resposta à infecção, são desencadeados mecanismos de reconhecimento específico do agente infeccioso, células de memória, indução da regulação da temperatura corporal, anorexia e outras respostas comportamentais.

Após identificar a natureza do patógeno e a ação das respostas imunes inatas, que atuam para limitar a propagação da infecção, se estabelece condições que estimulam a imunidade adaptativa. Essas respostas imunes eliminam quaisquer patógenos remanescentes e formam uma memória imunológica para ajudar a controlar rapidamente as reinfecções. O desenvolvimento da imunidade inata requer energia e substratos bioquímicos, entretanto, esses custos são menores, pois o processo de desenvolvimento é relativamente gradual e eficiente. O desenvolvimento da imunidade adquirida também requer energia e substratos bioquímicos, para alimentar a geração de um grande conjunto de linfócitos com diversas especificidades de ligação ao antígeno. Um custo adicional de desenvolvimento da imunidade adquirida é o tempo devido ao estabelecimento da memória imunológica. Portanto, os benefícios de respostas secundárias mais rápidas e eficazes são percebidos apenas com exposições subsequentes aos mesmos patógenos, enquanto as

respostas imunes adquiridas são mais lentas e menos eficazes na exposição inicial (McDade et al., 2016).

Quando se trata do sistema imunológico adaptativo, os mecanismos potenciais que medeiam os custos de aptidão, incluem imunopatologia na forma de dano tecidual crônico por respostas autoimunes e o custo energético extra de outra maquinaria fisiológica para reparo e reposição do tecido afetado (Råberg et al., 1998). No entanto, uma abordagem de otimização para a evolução do sistema imune adaptativo deve levar em consideração que a defesa imune inata já estava operando no início da defesa adaptativa. Como a defesa inata também deve ser cara em termos de energia, os custos líquidos da defesa adaptativa dependem de ela assumir ou não algumas das funções da defesa inata (Fearon e Locksley, 1996).

A infecção requer uma mudança no metabolismo, e essas mudanças diferem entre aguda e crônica ou prolongada (Figura 3). Durante a infecção aguda, a produção rápida de ATP em células imunes é necessária. As células imunes (linfócitos e leucócitos) mudam da fosforilação oxidativa para uma maior dependência da glicólise aeróbica para produção rápida de ATP (Kelly e O'Neill, 2015). Essa mudança é a favor da produção precipitada de ATP, enquanto a eficiência catabólica é prejudicada, resultando em diminuição da função mitocondrial e no aumento da produção de lactato.

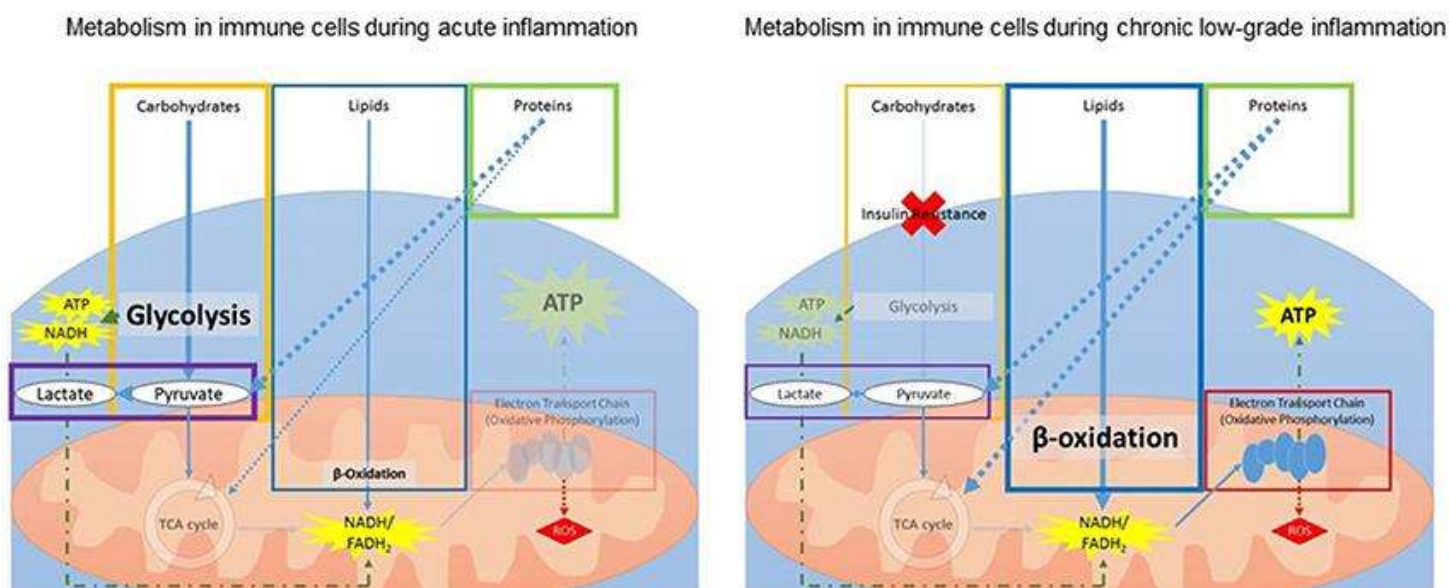


Figura 3. Descrição geral das alterações no metabolismo e uso dos nutrientes dentro das células imunes durante a infecção aguda (esquerda) e crônica (direita) (Lacourt et al., 2018).

Durante a infecção aguda, as células imunes aumentam a glicólise enquanto diminui o ciclo do ácido tricarboxílico (TCA) para geração rápida de ATP. Durante a infecção crônica de baixo grau, a resistência à insulina leva a uma diminuição na captação de glicose e glicólise pelas células imunes e, conseqüentemente, à menor produção geral de ATP a partir da glicose (Asghar e Sheikh, 2017). Portanto, durante a infecção de baixo grau em andamento, a energia e a glicose são desviadas de outros sistemas para dar suporte à resposta imune. Para compensar, o corpo aumenta o metabolismo de lipídios e proteínas para a produção de ATP (Liu et al., 2012). O metabolismo de lipídios é um processo lento e, portanto, não pode responder às rápidas necessidades de energia. Além disso, o aumento da dependência do metabolismo de proteínas significa que outras funções, como a promoção da estimulação do crescimento, são comprometidas (Le Floch et al., 2004).

A infecção, como outros processos estressores, leva ao aumento de espécies reativas de oxigênio (ROS) como resultado dos patógenos invasores (Maitra et al., 2012). No entanto, as ROS podem ser prejudiciais às mitocôndrias e ao DNA mitocondrial (Sinha et al., 2013) e, portanto, a exposição prolongada as ROS durante a infecção crônica, contribui para a disfunção mitocondrial, sendo este o principal responsável pelo estresse oxidativo, que contribui para mudanças funcionais nas células imunes (Sun et al., 2020). O estresse oxidativo surge de um desequilíbrio entre a produção de ROS e a capacidade do corpo de desintoxicar esses intermediários reativos ou reparar os danos resultantes. Diante disso, a febre decorrente da infecção, aumenta a taxa metabólica, levando ao aumento da atividade mitocondrial e, subseqüentemente, à maior produção de ROS. Embora o ROS seja essencial para a destruição de patógenos, seu acúmulo excessivo pode levar a danos oxidativos em células e tecidos. Isto requer um equilíbrio delicado entre uma resposta imunitária eficaz e a prevenção do stress oxidativo excessivo, mediante a participação de agentes antioxidantes.

Mecanismos moleculares da febre

A febre, definida como hipertermia regulada na temperatura corporal, faz parte da reação fisiológica a patógenos invasores, conhecida como resposta de

fase aguda ou não específica. Durante a febre, o sistema termorregulador é ajustado para favorecer a retenção de calor metabólico, enquanto a perda de calor é reduzida. A febre é frequentemente a primeira manifestação física de um sistema imunológico inato ativado e, como tal, serve como um sinal precoce sobre a presença de infecção ou inflamação (Blatteis et al., 2000).

O componente termorregulador da resposta imune a um patógeno pode ser um custo chave no orçamento energético de uma ave, quando a resposta desencadeada é a febre (Marais et al., 2011). A febre é caracterizada pela temperatura corporal elevada ativamente estabelecida e defendida por mecanismos de produção e/ou conservação de calor, que consistem em um importante auxiliar da função imunológica, protegendo o hospedeiro contra infecções por patógenos (Gray et al., 2013). O elevado custo da resposta febril, representa um desafio metabólico que pode exceder os seus benefícios em circunstâncias de aptidão fisiológica reduzida (Garami et al., 2018).

Embora o processo inflamatório causado pela inoculação de altas doses de endotoxina desencadearia um estado febril, ele resulta em uma diminuição da temperatura corporal, caracterizando um estado de hipotermia (Akarsu e Mamuk, 2007). A queda de temperatura corporal durante o choque endotoxêmico pode ser considerada uma resposta regulada, conhecida como anapirexia. Este fenômeno visa inibir a ocorrência de hipóxia tecidual e desempenha um papel defensivo (Corrigan et al., 2014), com intuito de economizar energia para defender os sistemas vitais 'tolerando' a presença patogênica, resposta que também é considerada benéfica para o hospedeiro (Amaral-Silva et al., 2021). Ao investigar os mecanismos moleculares da febre em resposta a um desafio patogênico, a metabolômica surge como uma ferramenta crucial, fornecendo informações valiosas sobre as alterações metabólicas associadas a esses eventos.

Metabolômica e Desafio Sanitário

O metabolismo é uma parte fundamental da fisiologia molecular, elucidar a estrutura e a regulação das vias metabólicas é crucial para obter uma perspectiva abrangente da função celular e compreender os mecanismos subjacentes às suas disfunções frente a um desafio sanitário. Diante disso, a metabolômica tornou-se uma ferramenta acessível e intensamente utilizada, que

revela a composição e alterações metabólicas (Chung et al., 2018). É possível observar as mudanças nas vias metabólicas, relacionadas a partir de diferenças nos perfis metabólicos, como aqueles relacionados às vias metabólicas de lipídios, energia e aminoácidos, fornecendo uma linha para as transições fisiológicas e metabólicas do hospedeiro (Afrouziyeh et al., 2022).

Os dados gerados fornecem uma informação do estado metabólico do organismo, refletindo mudanças nas vias metabólicas quando as aves são expostas a desafios patogênicos. Dois aspectos críticos destas alterações metabólicas são a renovação proteica (turnover proteico) e o metabolismo energético, que estão intimamente interligados. A quebra de proteínas em aminoácidos não só fornece substratos para a síntese de proteínas imunológicas, mas também o esqueleto carbonado é utilizado como fonte de energia. Os aminoácidos liberados da proteólise são desaminados e seus esqueletos de carbono entram no ciclo do TCA como intermediários. Por exemplo, a degradação de valina, leucina e isoleucina produz acetil-CoA e succinil-CoA, que são essenciais para a produção de ATP (Monirujjaman e Ferdouse, 2014). O ATP gerado a partir do aumento da glicólise, oxidação de ácidos graxos e atividade do ciclo TCA, são regulados positivamente para atender às crescentes demandas energéticas devido à ativação imunológica e aos processos de reparo tecidual (Soto-Heredero et al., 2020). Níveis aumentados de intermediários como piruvato, lactato e metabólitos do ciclo TCA indicam aumento da atividade metabólica e produção de energia (Martínez-Reys e Chandel, 2020).

Os aminoácidos desempenham um papel crucial na função imunológica e na reparação de tecidos. A análise metabolômica evidencia alterações nos níveis de aminoácidos, incluindo aqueles envolvidos na síntese da glutathione, um antioxidante essencial (Aoyama e Nkaki, 2015). O metabolismo lipídico também é significativamente afetado, as respostas inflamatórias muitas vezes levam a alterações nos perfis lipídicos, incluindo alterações nos fosfolipídios, ácidos graxos e ácidos biliares (Khovidhunkit et al., 2004). O aumento dos produtos da peroxidação lipídica indica estresse oxidativo e danos às membranas celulares.

A produção de ROS é elevada em resposta a desafios patogênicos, levando ao estresse oxidativo. O perfil metabolômico destaca níveis aumentados de antioxidantes como glutathione e ascorbato, refletindo a ativação de

mecanismos de defesa (Noctor et al., 2015). A via das pentoses fosfato é regulada positivamente para produzir NADPH, necessário para regenerar a glutatona reduzida e manter o equilíbrio redox (Fuentes-Lemus et al., 2023).

Portanto, com a análise de metabólitos em fluidos corporais e tecidos, frente ao desafio patogênico, é possível compreender a composição e as alterações desses metabólitos, e assim compreender como estes podem refletir as condições e padrões metabólicos do hospedeiro, que ajudam a descobrir ou interpretar potenciais mecanismos biológicos (Mozzi et al., 2013). A análise de perfis metabolômicos é uma ferramenta importante para gerar uma impressão digital metabólica celular sob condições de desafio. As alterações fornecem conhecimentos novos e significativos para o ajuste do metabolismo das células para contrabalancear o déficit energético. Ao identificar biomarcadores metabólicos específicos, é possível monitorar a progressão da doença, o tipo de desafio e a eficácia das intervenções, melhorando a saúde e a produtividade dos frangos de corte.

Modelos de Desafio: Lipopolissacarídeo vs. Eimeria + Clostridium

Diferentes modelos de desafio são abordados na literatura, a abordagem mais comum tem sido a utilização de vários padrões moleculares associados a patógenos (PAMPs), nomeadamente componentes inatos de patógenos específicos, para estimular o sistema imunológico e investigar processos resultantes. O PAMP mais utilizado é o lipopolissacarídeo (LPS), que é um componente celular isolado de bactérias Gram-negativas (Figura 4) e, portanto, simula a infecção bacteriana, sendo uma endotoxina usada como modelo para estresse imunológico em animais (Sampath, 2018), ele é composto por proteína, oligossacarídeo, antígeno O e pelo componente bioativo tóxico que é reconhecido pelo organismo na resposta imune, o Lipídeo A. O LPS injetado por via abdominal ou oral pode desencadear uma resposta ao estresse oxidativo, levando ao acúmulo de ROS, que induz uma resposta inflamatória aguda, reconstrói o suprimento de nutrientes, impede a taxa de crescimento, e causa respostas inflamatórias sistêmicas (Han et al., 2020).

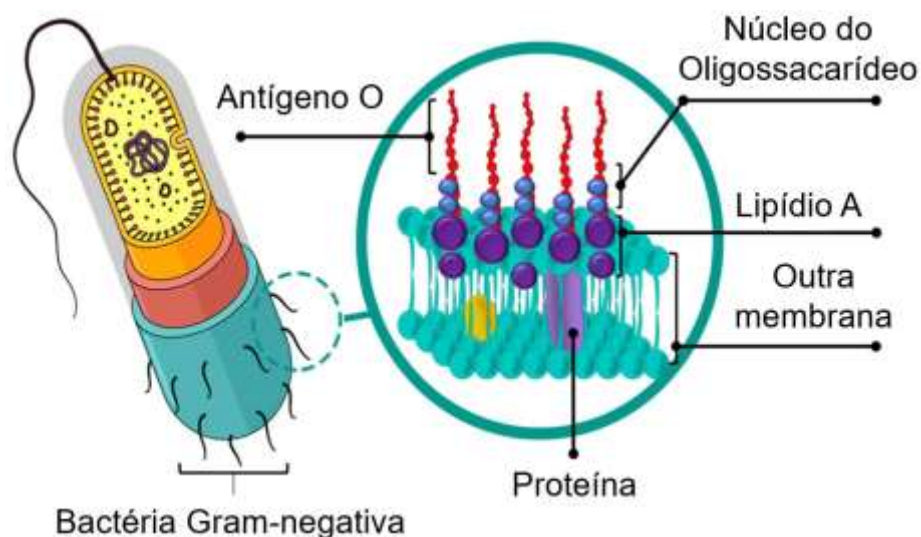


Figura 4. Componentes do Lipopolissacarídeo (Marcano et al., 2021).

Já a infecção por algum tipo de patógeno, como o protozoário *Eimeria* apresenta uma patogênese que inicia com a destruição dos enterócitos da mucosa intestinal do hospedeiro, resultando em aumento da permeabilidade celular, vazamento de nutrientes e proteínas plasmáticas, digestão e absorção prejudicadas, contribuindo para os efeitos clínicos e subclínicos da coccidiose (Yang et al., 2020). Isso compromete a homeostase intestinal do hospedeiro, causando má absorção, redução da conversão alimentar e ganho de peso (Leung et al., 2018). O grave dano intestinal causado pela colonização de *Eimeria* não afeta apenas as células epiteliais, mas causa grande perturbação das comunidades microbianas intestinais no trato gastrointestinal, promovendo a colonização e proliferação de outros patógenos, sendo o principal o *Clostridium perfringens* que é uma bactéria Gram-positiva, que produz toxinas formadoras de poros que rompem a bicamada da membrana fosfolipídica de células causando um influxo de íons (ou seja, Na^+ , Cl^- , Ca^{2+} , etc.) que pode levar à lise osmótica das células (Alouf, 2001), provocando lesões ulcerativas e necrose da mucosa no intestino, induzindo assim, uma resposta inflamatória crônica.

O *Clostridium* atua em conjunto com a *Eimeria*, como principal fator predisponente da enterite necrótica, a coccidiose pode facilitar a colonização e proliferação dele devido ao dano da camada epitelial intestinal (Madlala et al., 2021). O ciclo de vida da *Eimeria* começa com a ingestão de oocistos esporulados (maduros), na moela os oocistos se rompem, liberam os esporocistos e com a ação enzimática (sais biliares e a quimotripsina), estimulam

a liberação dos esporozoítos do oocisto. Uma vez liberados, os esporozoítos invadem as células intestinais iniciando o estágio de desenvolvimento assexuado, chamado esquizogonia. Após um número variável de ciclos assexuados, os gametas são formados e o estágio sexual de desenvolvimento começa (gamogonia). A fase sexual termina com a produção e liberação de oocistos no lúmen intestinal, os oocistos devem esporular para se tornarem infecciosos. O processo de esporulação geralmente leva de 2 a 3 dias dependendo das condições ambientais (Waldenstedt et al., 2001). Uma vez que os parasitas *Eimeria* perturbam a integridade do epitélio, induzem o aumento das secreções de muco intestinal, ricos em nutrientes favoráveis à replicação do *C. perfringens* e também um ambiente anaeróbico propício a este. Sendo assim, ambos microrganismos são os principais responsáveis de ocasionar a enterite necrótica, uma doença que afeta a produção de frangos de corte e ocasiona elevados prejuízos.

Portanto, ambos os modelos de indução de desafio ocasionam perturbações na saúde das aves, porém em amplitudes diferentes (Tabela 1).

Tabela 1. Principais diferenças entre os modelos de indução de desafio sanitário.

Lipopolissacarídeo	Enterite Necrótica
Inflamação Sistêmica	Inflamação Crônica
Reação Imunológica Inata (Resposta Imune Inespecífica)	Reação Imunológica Adaptativa (Resposta Imune Específica)
Inflamação aguda: é a primeira linha de defesa contra um patógeno ou lesão.	Inflamação crônica: ocorre quando a resposta imune não consegue eliminar a causa da resposta imune ou da inflamação aguda.
Início agudo: A inflamação sistêmica normalmente tem início rápido e costuma ser uma resposta a infecções, lesões ou outras ameaças imediatas.	Início gradual: A inflamação crônica se desenvolve lentamente e pode persistir por meses a anos.
Curta duração: Geralmente dura um curto período, muitas vezes resolvendo dentro de dias a semanas.	Longa Duração: É uma resposta inflamatória prolongada que não se resolve rapidamente.

Ativação generalizada: Envolve a ativação do sistema imunológico em todo o corpo, levando a sintomas generalizados.	Localizada ou Sistêmica: Pode ser localizada em tecidos ou órgãos específicos, mas também pode afetar todo o corpo.
Liberação de citocinas: Há uma liberação rápida de citocinas pró-inflamatórias como IL-1, IL-6 e TNF- α , que podem afetar vários órgãos.	Ativação imunológica persistente: Há uma ativação contínua das células imunológicas, levando a danos nos tecidos e ciclos de reparação.

O LPS é um modelo de desafio associado à resposta inflamatória sistêmica, ou seja, uma infecção localizada onde a endotoxina vai direto a corrente sanguínea, ultrapassando as barreiras físicas de proteção do organismo como a mucosa, barreira celular, entre outras. O período de resposta é mais curto, já que todo processo de infecção bacteriana, como liberação de exo e endotoxinas é minimizado, finalizando na inflamação sistêmica. Entretanto o desenvolvimento da infecção bacteriana requer um longo processo até entrar na corrente sanguínea e prejudicar todo o organismo. Os desafios com LPS, embora não sejam um desafio bacteriano 'verdadeiro', simulam um tipo de resposta semelhante, mas de magnitude menor aos desafios bacterianos (Sandberg et al., 2007).

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CAPÍTULO 2 - Dynamics of energy utilization in growing chicks under challenge conditions

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ENERGY UTILIZATION IN CHICKS UNDER CHALLENGE

Dynamics of energy utilization in growing chicks under challenge conditions

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ABSTRACT

The health challenges in broiler chickens significantly impact poultry production, particularly through reduced feed intake and adverse effects on nutrient and energy digestibility and utilization. This study aimed to assess the energy utilization dynamics in growing broiler chickens under pathogenic challenge by *Eimeria maxima* (coccidiosis) and *Clostridium perfringens* to describe the energy utilization to support the immune response. A total of 96 one-day-old male Cobb-500 broiler chickens were reared in cages until they were 12 days of age. At 13 days of age, the birds were randomly distributed to the experimental treatments: **AL** (*ad libitum* feed intake with no challenge), **C** (challenged birds), and **PF** (pair feeding to match the feed intake of the **C** group without challenge). The challenge protocol involved inoculating the **C** group with 1 mL of *E. maxima* (99,000 oocysts/ml) at 14 days of age, followed by inoculation with *C. perfringens* (2.5×10^6 CFU) at 18, 19 and 20 days of age. Performance and energy utilization were evaluated from 14 to 22 days. The results demonstrate that the **AL** treatment showed a higher feed intake and body weight than the **PF** and **C** treatments ($P < 0.05$). Pathogenic challenge notably influenced energy utilization, with a significant reduction in metabolizable energy intake ($P < 0.05$), and increase mobilization of endogenous energy retained as fat ($P < 0.05$). The decrease in residual energy efficiency was mainly attributed to the specific effect of the challenge (approximately 20%) and, to a lesser extent, to the anorexic effect of the disease (approximately 10%). These findings suggest that under pathogenic conditions, broiler chickens reduce feed intake, which affect body weight. However, they require substantial energy to maintain essential functions and activate immune responses. Notably, during this period of anorexia, energy utilization characterized by reduced retention and increase mobilizing energy storage as fat. Understanding these responses is

crucial for developing strategies to optimize growth in broiler chickens under challenging conditions.

Key words: *Clostridium perfringens*; *Eimeria maxima*; Energy metabolism; Growing broiler; Heat production.

INTRODUCTION

Poultry production continually advances by incorporating innovations in nutrition, health, and animal welfare technologies. The breeding of broiler chickens for enhanced growth rate has significantly reshaped the development of their digestive system, yielding adverse consequences such as metabolic disruptions, compromised immune function, and diminished resistance to pathogens (Zuidhof et al., 2014). The swift growth exhibited by modern broiler chicks is associated with their ability to consume a large quantity of feed combined with an efficient utilization of nutrients. Disruption of the animal's health can trigger a reduction in feed intake (**FI**) or anorexia, leading to decreased nutrient and energy utilization (Taylor et al., 2022), with impact on the immune response and further detrimental effects (Arai, 2014; M'Sadeq et al., 2016).

The impact of a health challenge on energy utilization and the subsequent effect on performance depends on many factors, including the type of pathogenic agent, disease severity, and physiological state of the animal (Klasing, 2007; Remus et al., 2014). The metabolic response following infection generally adheres to a distinctive pattern characterized by a decrease in FI and can be divided into three phases: 1) prepatent, 2) acute stage of infection, and 3) recovery (Taylor et al., 2022). Consequently, understanding these changes in FI of birds subject to a sanitary challenge and the metabolic adaptation in energy utilization stands as an important pursuit.

Several challenge models have been proposed to induce a pathogenic challenge under experimental conditions that reflect practical poultry diseases. One common model, entailing the exposure of birds to *Eimeria* spp. and *Clostridium perfringens*, elicits the symbiotic interaction that compromises gut integrity and hinders passage rate, resulting in reduced nutrient absorption and consequently in nutrient and energy utilization (Collier et al., 2008; Bortoluzzi et al., 2019). Immune responses and tissue repair negatively affect

animal productivity (Klasing, 2009), and imply a high energy demand by the animal, increasing the total energy expenditure compared to a healthy animal. This requires to obtain immediate energy from the catabolism of reserve tissues, significantly reducing energy retention (Adedokun and Olojede, 2019).

Immune system disturbances reduce protein and fat anabolism while enhancing the catabolic process for synthesizing immune effector molecules (Yu et al., 2016). There is a shift in partitioning dietary energy away from skeletal muscle growth towards supporting metabolic processes that facilitate responses that support the immune system. In response to immune stress, the metabolic priorities of birds undergo rearrangement, resulting in energy taken away from muscle protein deposition and growth to support the upregulation of the immune response (Johnson, 1997; Klasing, 2007).

During the initial stage of infection, up to 70% of the reduction in performance can be attributed to decreased FI, while the remaining 30% is ascribed to malabsorption and compromised gut health status (Klasing et al., 1987). Nonetheless, the impact of anorexia and the utilization of endogenous energy sources during disease periods on energy utilization for growing birds remains unclear. In this sense, the damage caused by pathogens and their anorexic effect occur simultaneously. These phenomena characterize the stage of the sanitary challenge, corresponding to the pathogen's infection process and the animal's immune response. Both events can be described as dynamic processes, with their description being essential in terms of energy utilization.

Based on that, understanding the dynamics of energy utilization in challenged broiler chickens involves several important steps. Firstly, when faced with health challenges such as anorexia, there is a notable shift in energy utilization to prioritize essential functions and activation of the immune system (Taylor et al., 2022). This phenomenon is exacerbated by the dependence on endogenous energy sources, leading to

decreased tissue deposition and growth rates (Murray and Murray, 1979). Consequently, unraveling the dynamics of energy utilization becomes crucial as animal metabolism adapts to achieve the extra energy demands during the disease's development. To address these complexities, this study aimed to describe the stages of energy utilization in growing chicks under pathogenic challenges and evaluating how these challenges influence on FI, body weight (**BW**), and energy utilization, thus improving our understanding of metabolic adaptations in birds during challenge conditions.

MATERIALS AND METHODS

Ethical Implications

All animal procedures and management were approved by The Animal Care and Use Committee of the School of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil, under protocol number n°.5399/20.

Animals and Experimental Design

This study was carried out at the Poultry Science Laboratory (LAVINESP) of São Paulo State University, Jaboticabal – SP, Brazil. A total of 96 one-day-old male Cobb-500 broiler chickens were used, divided into three treatments of eight birds each, with four replications per treatment. The birds were raised in cages of dimensions 1.2 m² each, until reaching the data collection period at 12 days of age. The experiment occurred under thermoneutral conditions, maintaining an ambient temperature of approximately 26°C and relative humidity of 65%.

At 13 days of age, the birds were randomly allotted to experimental treatments, based on the same average of BW for the treatments. The treatments were designed as follows: **AL** (non-challenged birds with *ad libitum* FI), **C** (challenged birds involving *E.*

maxima and *C. perfringens* with *ad libitum* FI), and **PF** (non-challenged birds, and pair feeding to match the FI of the **C** group measured one day earlier).

As shown in Table 1, a diet was formulated aimed to meet the nutritional requirements of growing birds, following the recommendations of Rostagno et al. (2017).

Animal Management and Challenge Protocol

At 12 days of age post-hatch, the broiler chickens achieved an average BW of 390 $\pm$ 60 g. Throughout the pre-experimental period, the birds were housed in climate-controlled conditions, ensuring *ad libitum* access to both water and feed. The lighting program used to a 18L:6D for the entire experimental period. On the 13th day, the birds were transferred to open-circuit respirometry chambers for a one-day habituation period. Subsequent measurements and data were collected when the birds were between 14 and 22 days old. The outlined protocol and data collection procedure are detailed in Figure 1.

At the start of the experimental period at 14th day, the birds assigned to the **C** treatment underwent oral inoculation by gavage with 1 mL of *E. maxima* solution (99,900 oocysts/ml – quantified through the oocyst count method as described by Gordon and Whitlock, (1939). Then, at 18, 19, and 20th day, these birds received oral inoculation of 2.5×10^6 colony-forming units of *C. perfringens* (Type G - NetB), four days after *E. maxima*. Birds in the **AL** and **PF** treatments received 1 mL of saline solution by gavage during inoculation days to standardize the handling stress. The challenge protocol used was adapted according to those reported in the literature (Lu et al., 2020; Nicholds et al., 2021; Feng et al., 2022; and Goo et al., 2023).

Between 14 to 22 days of age, FI (kg/bird/d), BW (kg), cumulative feed intake (**CFI**), and gas exchanges (oxygen consumption – $\dot{V}O_2$ and carbon dioxide production – $\dot{V}CO_2$) were recorded daily. Measurements of $\dot{V}O_2$ and $\dot{V}CO_2$ were performed continuously for 23 hours per day. The total excreta collection procedure from each

chamber was conducted daily. Excreta sampling was identified by day, storage at -20°C, and representative subsamples were acquired following thorough mixing and drying for subsequent analysis. For the pair feeding in treatment **PF**, the average FI of the **C** treatment was calculated daily and the same quantity was given to the birds in treatment **PF** the next day.

Indirect Calorimetry

The indirect calorimetry system consisted of six open-circuit chambers with dimensions of 90×85×95 cm, was calibrated as described by Riveros, et al. (2023). Each chamber had a temperature control system and was coupled to the Sable System Classic Line components (Sable System, NV, USA) for gas flow, sampling, and analysis. Environmental air was drawn into the chamber using negative pressure generated by the flow kit pump (FK-100, Sable System, NV, USA). Approximately 160 mL/min of air was sampled for analysis, and the water vapor pressure (RH, Sable System, NV, USA) was recorded. The air sample was then dried using calcium sulfite and subsequently conducted to analyze the oxygen (**O₂**) and carbon dioxide (**CO₂**) fractional concentrations to express the volumetric concentration for a gas volume corrected to standard barometric pressure. The gas concentrations in the air sample were analyzed using a paramagnetic analyzer for O₂ (PA, Sable System, NV, USA) and an infrared analyzer for CO₂ (CA, Sable System, NV, USA). Gas exchange measurements were recorded every second and averaged per minute to calculate heat production (**HP**). $\dot{V}O_2$, $\dot{V}CO_2$, and HP data were computed following the calculations described by Lighton (2018) for negative-pressure open-circuit system chambers.

The in-going airflow (F_{in} , L/min) was calculated as follows:

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

The $\dot{V}O_2$ (L/d) and $\dot{V}CO_2$ (L/d) were obtained as:

$$\dot{V}O_2 = F_{in} \times [O_2]_{in} - F_{out} \times [O_2]_{out}$$

$$\dot{V}CO_2 = F_{out} \times [CO_2]_{out} - F_{in} \times [CO_2]_{in}$$

Where F_{out} is the outgoing airflow, $[O_2]_{in}$, and $[CO_2]_{in}$ are the atmospheric gas concentrations. The HP (kcal/bird/d) was calculated by the Brouwer equations (1965):

$$HP \text{ (kcal)} = 3.86 \times \dot{V}O_2 + 1.20 \times \dot{V}CO_2$$

Energy and Nitrogen Efficiency

The diet and excreta samples were conducted to the laboratory for analyses: dry matter, total nitrogen content and gross energy value. Sample drying at 105°C for sixteen hours was carried out to determine the dry matter content. The total nitrogen content was evaluated using the Kjeldahl method (AOAC, 2005). Subsequently, the crude protein content was computed employing a conversion factor of 6.25 grams of crude protein per gram of nitrogen. Determination of the gross energy in the samples was achieved via complete combustion employing an adiabatic calorimeter (IKA C 2000 basic, USA).

The nitrogen retention (g/bird/d) and the efficiency of nitrogen utilization (**k_N**) were calculated from the determination of nitrogen intake and excreted. The apparent metabolizable energy intake (**ME_i**, kcal/bird/d) was calculated by the difference between the gross energy intake and excreted.

The components of energy partitioning, as energy retained (**ER**) and ER as protein and fat, were determined based on ME_i and HP. Energy retained as protein (**ER_{pt}**) was computed using the nitrogen-to-protein constant (6.25 g protein/g N) and the protein's caloric value (5.6 cal/g protein). The energy retained as fat (**ER_{fat}**) was obtained from the difference between ER and ER_{pt}.

Residual Energy Efficiency

The residual energy efficiency (**REE**) was determined to discern the energy intake due to the disease-specific or anorexic effect. A regression analysis was

performed, using the metabolic BW ($\text{kg}^{0.75}$) and daily body weight gain (**BWG**) as independent variables and the apparent metabolizable energy intake MEi as the dependent variable for the **AL** treatment:

$$MEi = b \times BW^{0.75} + c \times BWG$$

Where **b** is energy required for maintenance expressed per unit of metabolic weight ($\text{kg}^{0.75}$) and **c** is energy use per g of BWG. The REE was then calculated as:

$$REE = \frac{MEi_{obs} - MEi_{exp}}{MEi_{exp}}$$

Where MEi_{obs} is the measured energy intake (for treatment **C** and **PF**) and MEi_{exp} the expected energy intake derived from the regression equation.

Statistical Analysis

All data were analyzed using the statistical software SAS. Energy metabolism data, MEi, HP, ER, ERpt, and ERfat, were expressed in kcal per metabolic $BW^{0.75}$. The performance, energy metabolism, and nitrogen balance parameters were analyzed over three days, starting when the experiment began at 14 days of age (0 days post-infection, **DPI**). The subsequent days were grouped into three-day intervals (1-3, 4-6, and 7-9 DPI). This criterion was adopted to express energy partitioning and to align the HP and excreta collection variables. These variables underwent analysis employing a two-way analysis of variance (ANOVA), considering treatment and DPI as longitudinal factors. The statistical analysis was executed using PROC GLM. When interactions were observed, orthogonal contrast analyses were employed. Subsequently, treatments were analyzed for each DPI to elucidate the effects of the treatments for the same period. Statistical significance between means was determined using the Tukey test, employing a confidence level of 95%.

To determine the relative variation of REE, the fractional reduction in FI (anorexia) or the impact of diseases “itself” on energy utilization, a linear regression was

fitted exclusively for the **AL** treatment. This analysis describes the relationship of REE with ME_i, expressed per kcal/bird/d using PROC REG. The outcome elucidated the energy intake alterations resulting from reduced FI or the influence of disease on energy utilization.

RESULTS

Performance Response

The performance response of BW, FI, and CFI were shown that the FI during 4-6 DPI dropped on the **C**, in accordance with the *E. maxima* cycle and pathogenic action and the *C. perfringens* inoculation (Figure 2A). On the other hand, the **AL** treatment maintains an increasing pattern of FI along the evaluation periods. Sequentially, for the 7-9 DPI, the **C** treatments try to recover on the daily FI, achieving the same FI as **AL** ($P > 0.05$). These differences on the daily FI resulted in a variation of the CFI (Figure 2B), where the **C** and **PF** treatments reported significantly lower than **AL** from 4-6 DPI ($P < 0.05$). The same behavior was registered for the BW (Figure 2C), the **C** and **PF** treatments exhibited lower BW compared to the **AL** treatment ($P < 0.05$) at 4-6 DPI. At the end of the challenge (7-9 DPI), the BW of the **C** and **PF** groups remained lower than treatment **AL** ($P < 0.05$). This difference indicates that the growth of broiler chickens was significantly affected by the challenge, as well as by the reduction in FI during the last two periods.

Energy Balance

All energy metabolism parameters were expressed per unit of metabolic weight ($\text{kg}^{0.75}$). The energy metabolism variables, specifically, ME_i, HP, ER, ER_{pt} and ER_{fat} displayed a consistent pattern, with the **C** treatment demonstrating lower values than **AL** treatment. Differences in ME_i between treatments appeared on 4-6 DPI (Figure 3A), where the treatment **C** showed the lowest value ($P < 0.05$), and the treatments **AL** and **PF**

reported the higher values ($P > 0.05$).

The most significant difference in HP between all treatments was observed at 4-6 DPI, as shown in Figure 3B. Treatment **C** presented HP values lower than **AL** ($P < 0.05$) and treatment **PF** was the same to treatment **AL** and **C** ($P > 0.05$).

The differences in ER between treatments emerged 4-6 DPI, indicating a drop of ER of the bird of the treatment **C** showed lower ER than **AL** and **PF** ($P < 0.05$), given in Figure 3C. In the same period (4-6 DPI), treatment **C** exhibited lowers ERpt and ERfat than **AL** ($P < 0.05$), Figure 3D e 3E, respectively. However, the group **PF** was the same as treatment **AL**, and **C** ($P > 0.05$). Importantly, ERfat was present negative values in **C** treatment.

Nitrogen Efficiency

N intake exhibited a pattern similar to FI, where **C** and **PF** treatments showed lower N intake ($P > 0.05$) than **AL** treatment ($P < 0.05$) in the 4-6 DPI period. At 7-9 DPI the **C** treatments showed a recovery of N intake but being similar to **PF** birds (Figure 4A). As shown in Figure 4B, significant variation was evident in N excreted between treatments, with pronounced disparities detected also in the 4-6 and 7-9 DPI periods. Specifically, group **C** exhibited greater excretion (1.07 g/b/d) but same ($P > 0.05$) to the **AL** treatment (0.98 g/b/d), and the **PF** treatment recorded the lowest ($P < 0.05$) N excretion (0.87 g/b/d) compared to the other groups.

As shown in Figure 4C, the most variation between treatments for the k_N was in the period of 4-6 DPI, in which the k_N values were same between the **AL** and **PF** treatments ($P > 0.05$), and markedly lower from the treatment **C** ($P < 0.05$). The **AL** treatment showed the highest efficiency (0.71), similar to the **PF** treatment (0.69), while the **C** treatment showed the lowest nitrogen utilization efficiency (0.65) with a drop of k_N of around 0.55 at 4-6 DPI.

Residual Energy Efficiency

The REE was conducted to ascertain the projected energy intake, as predicted by the **AL** treatments. This predicted energy intake is derived from the interplay of metabolic BW and BWG among healthy birds reared under adequate conditions. The disparity of the REE as a fractional partitioning for the **PF** and **C** groups is illustrated in Figure 5A and 5B, respectively. The **AL** treatments exhibited a marginal fluctuation in REE, falling within the range of less than 5%, which is considered a normative variance within experimental units.

Analyzing the **PF** treatments, a reduction in REE was evident at 5, 6, and 7 DPI, with a substantial decline of 20.5% in REE on day 5 DPI. Conversely, the **C** treatments showed a decrease from days 4 to 7 DPI, with the most substantial reduction on day 5 DPI of 33.1%. This reduction persisted on day 6 DPI at 27.2%. Similar to other variables, the decline in REE could potentially impact energy utilization, particularly on the energy retention, during the pronounced at 5 DPI. Furthermore, on 5 DPI, the primary reduction is attributed to the disease's effect (Figure 5B), with a less influence from anorexic factors (Figure 5A). This suggests that diseases have a more pronounced impact on voluntary energy intake reduction. By 6 DPI, both the **PF** and **C** treatments demonstrated equivalent reductions in REE, averaging 9.4%.

DISCUSSION

This study investigated the dynamics of energy utilization and the variations attributable to the anorexic effects induced by a pathogenic challenge in broiler chickens. The pathogenic organisms used, *E. maxima* and *C. perfringens*, induce gut damage, resulting in reduced FI. The anorexia observed influences nutrient utilization and broiler chick growth. The classical challenge model used in this study demonstrates a moderate

disease response, primarily manifesting as a reduced FI, leading to decrease growth rate. Notably, no mortality was register due to the challenge.

The overall average decline in FI resulting from the health challenge induction in growing chickens has been previously investigated by Amerah and Ravindran (2015), who documented an 8.5% reduction in FI during the evaluation phase of broiler chickens at 1-21 days of age (infected at 14 days of age). Our findings are similar, revealing a comparable reduction of approximately 9.4% (**C** vs **AL** treatments). However, a previous study by Parker et al. (2007) reported a marked 21% reduction in FI among broiler chickens in the same age range. Discrepancies between these investigations may stem from variations in challenge severity, the agent's pathogenic potential, the birds' physiological status, and other factors. Parker et al. (2007) employed multiple strains of *Eimeria* spp; including *E. acervulina*, *E. maxima*, and *E. tenella* at higher doses of 2×10^5 , 1×10^5 , and 1×10^5 viable oocysts/ mL, respectively. Conversely, the genetic strain of the broiler chicken subjects could manifest an elevated resilience against coccidiosis. Moreover, it is pertinent to consider that dietary nutrient adequacy may play a pivotal role in challenge-induced issues, as suggested by this research. This underscores the intricate interplay between genetic factors, dietary nutritional contribution, and the manifestation of challenge-associated problems.

The decline in FI inevitably leads to a concurrent reduction in the BW of the challenged birds. Earlier investigations have documented daily BW gain reduction by 17.8% (Amerah and Ravindran, 2015). This observation closely aligns with our study, wherein a BW gain decreased by 22% (53.6 and 42.0 g/bird/day for **AL** and **C** treatments, respectively) over the entire experimental period. Furthermore, at 4-6 DPI, the difference between the **AL** and **C** treatments amounted to 10%, Parker et al. (2007) reported a 45% reduction in BW in chickens subjected to the coccidiosis challenge seven DPI.

In addition to the decline in voluntary FI and the anorexic impact resulting from the disease effect, this study introduces other relevant factors related to the nutrient malabsorption induced by the pathogenic agents, causing physical damage to the gut epithelium and enterocyte disruption (Shini et al., 2021). Furthermore, the birds in the PF treatment exhibit a similar BW trend to those in the C treatment, highlighting the efficacy of the pair feeding strategy in isolating the FI reduction from the health challenge effect.

The pattern of weight gain reduction at specific DPI ages aligns with the findings of Bortoluzzi et al. (2019) and Akerele et al. (2022), who investigated the effects of challenges on BW in broiler chickens and observed transient setbacks in weight gain. The challenge model had a notable impact on FI throughout the DPI period, revealing a reduction in FI five days after inoculation, which persisted for the following three days. This observation is consistent with previous reports (Amerah and Ravindran, 2015; Parker, 2017; Sakkas et al., 2018) that documented FI depression around five DPI and maintained under reduced FI for three to five more days. This timeframe is sufficiently long to potentially compromise the BW and tissue deposition in the challenged broilers.

When employing the challenge model involving *E. maxima*, the observed effects can be attributed to this pathogen due to its intricate involvement in its life cycle, which spans approximately six days (Reid, 1973). Conversely, Nicholds et al. (2021), in a combined challenge involving both *E. maxima* and *C. perfringens*, noted a more pronounced decline in BW seven DPI of *E. maxima* and *C. perfringens*. Our study revealed a more substantial reduction in FI between days 4 and 6 DPI. These attributes potentially amplify the susceptibility of birds in the current experimental design challenge model, wherein challenged birds exhibited a significant decrease in both FI and BW from days 4 to 6 following *E. maxima* inoculation.

Another important aspect requiring evaluation pertains to the post-inoculation

recovery of both FI and BW in broiler chickens. The duration of this recovery phase varies and is primarily contingent upon the rearing conditions provided for the birds. An additional determinant is the physiological state of the broiler chick population, intricately linked to genetic predisposition and robustness in overcoming challenging health conditions (Bailey et al., 2018). Consistent with this finding, Tompkins et al. (2023) reported that the disparity in growth between the challenge and non-challenge groups was due to the metabolic cost of fever induction, immune activation, oxidative stress, and other pertinent factors. These findings significantly contribute to our understanding of the impact of *Eimeria* challenge on FI dynamics and energy utilization, evidencing an intricate interplay between health challenge, metabolic processes, and growth dynamics in broiler chickens submitted to pathogenic challenge.

Broiler submitted to PF and C treatment exhibited similar feed intake throughout the experimental period, owing to the implementation of pair feeding protocol. By examining the energy partitioning between these groups, we can gain insight into whether the voluntary reduction in energy intake can be attributed to the anorexic effect of the diseases. These findings align with FI patterns where unrestricted access to feed (AL treatment) contributed to the observed increase in energy intake. Humphrey and Klasing (2004) provided evidence that disease-induced immunologic stress profoundly impacts general metabolic processes, affecting both tissue deposition and energy expenditure as metabolic heat.

According to M'Sadeq et al. (2016), lower FI and metabolic disturbances can directly impact the energy availability for birds, consequently affecting HP, as evidenced by differences observed between challenged and non-challenged birds. Birds with free access to feed produce more heat, due to digestion and metabolization of higher amount of feed than challenged birds or with feed restriction, related with the amount of feed

ingested. Immunologic stress induced by disease challenges induce of appetite deprivation, and decreased the physical activity, a non-specific behavioral mechanism to external protection and energy saving for other essential function. The noteworthy disparity in HP among treatments on 4-6 DPI indicates that feeding conditions and challenges can influence HP in broiler chickens. Similar findings were reported by M'Sadeq et al. (2016), highlighting a higher HP in birds fed *ad libitum* due to increased energy intake and physical activity associated with unrestricted feeding, in addition to metabolic reactions of absorption and digestion. In the same way, the **PF** birds evidenced a lower HP at 4-6 DPI, since these birds were maintained under a restricted feed regimen, reducing the thermal effect of lower amount of feed and lesser physical activity, directly impacting the total HP (Horna et al., 2023).

The greater variation in energy intake observed in challenged birds can be attributed to the individual response variability within each group, influenced by the physiological status of each experimental unit. Zhang et al. (2016) underscored differences between individual and group observations, with each individual displaying varying responses to the challenge based on their physiological status.

Furthermore, there was an early decrease in ER (4-6 DPI) for the challenged birds, indicating that these birds experienced a reduction in energy retention before energy intake decreased due to the anorexic effect of challenge. The health challenge condition can impact both energy retention and maintenance. The maintenance energy requirement of broiler chickens may increase due to immune system activation and the energy expenditure associated with combating the challenge. Furthermore, a reduction in the digestibility of most dietary nutrients under these conditions could be suggested (Amerah and Ravindran, 2015; Diaz Carrasco et al., 2019).

Concerning ER_{pt}, none of the treatments yielded negative values, indicating that

protein retention persisted across all conditions, although reduction on the deposition rate of this tissue for challenge and **PF** birds. The discernible disparities in ERpt underscore the influence of feeding regimens and challenges on protein utilization and retention in broiler chickens. Fasina and Lillehoj (2019) reported that challenging broiler chickens with *C. perfringens* induced an inflammatory response in the intestine epithelium, leading to increased production of pro-inflammatory cytokines. This response requires of free-amino acid sources, ultimately affecting the muscle tissue syntheses. Additionally, *E. maxima* cause tissue damage to the mucosa and submucosa (Perez-Carbajal et al., 2010), thereby adversely influencing intestinal morphology, particularly villous shortening (Kettunen et al., 2001), and reducing digestive enzyme activities (Williams, 2005), and them requiring protein sources to repair the damaged tissues and increase the protein turnover.

The ERpt reduction indicate a lean tissue synthesis rate reduction, particularly on 4-6 DPI. A similar data was shown by Collin et al. (2003), indicating a reduction in protein and promoting an accretion of body fat when compared **PF** versus **C** treatment. Also, challenged birds need to allocate protein to both body maintenance and immunology response. In a challenging condition, the protein turnover rate is high due to continuous repair of the intestinal mucosa enterocyte.

Furthermore, the energy retention ERfat was severely diminished, with negative values observed in the challenged birds in the period 4-6 d postinfection. These findings could indicate that under challenge conditions, energy must be sourced from endogenous reserves to address the tissue repair and immunological response. Additionally, birds subjected to the **PF** treatment exhibited decrease ERfat values at 4-6 DPI, suggesting that the reduction in energy intake was lower, necessitating the utilization of endogenous sources for metabolic processes. These results suggest that birds with a proportional

reduction in FI comparable to animals under health challenges consumed energy below their requirement, forcing the mobilization of endogenous sources to meet their energy demands.

The initial reduction in REE was noted at 4 DPI, primarily attributed to the effect of the health challenge (**C** treatment); however, the anorexic effect, associated with the reduction in REE for birds in the **C** treatment, was observed at 5 DPI, indicating a “delayed” onset of the anorexic effect due to the health pathogenic. As shown in the figure 5 (A and B) at 5 DPI in the **C** treatment it was observed a reduction of 33.1% in the REE representing the effect of anorexia (20.5%) and pathogenic challenge (12.6%) when compared to **PF** treatment. By 6 and 7 DPI, recovery is evident, showing a reduction of less than 10% of REE. Meanwhile, the REE in the birds at 7 DPI cannot match between the challenge and non-challenge birds fed *ad libitum*, as the challenged birds are smaller in size due to the drop in BW, thus unable to intake the same quantity as their non-challenge counterparts.

CONCLUSION

The results of this work demonstrate that in challenging conditions, birds reduce feed intake, consequently affecting the growth. However, under these conditions, birds require energy to guaranty a continuous supply for immune response. The results also highlight those pathogenic challenges lead to a decrease in energy retention rates, and evidencing body fat tissue mobilization as energy source for immune response. Furthermore, the reduction in residual energy efficiency can be attributed mainly to the specific effect of the disease and, to a lesser extent, to the nonspecific anorexic effect. Understanding these responses to health challenges can help develop effective strategies to optimize bird growth. More research is needed to explore the specific mechanisms

underlying these responses and to implement specific interventions to improve poultry production.

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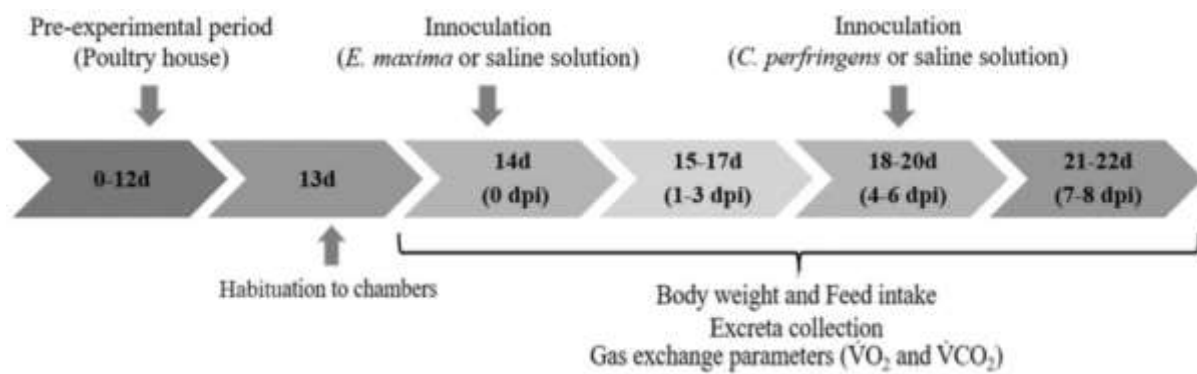


Figure 1. Experimental protocol for adaptation, gas exchange measurement, and data collection of growing broiler chickens subject or not to health challenge with *E. maxima* e *C. perfringens*.

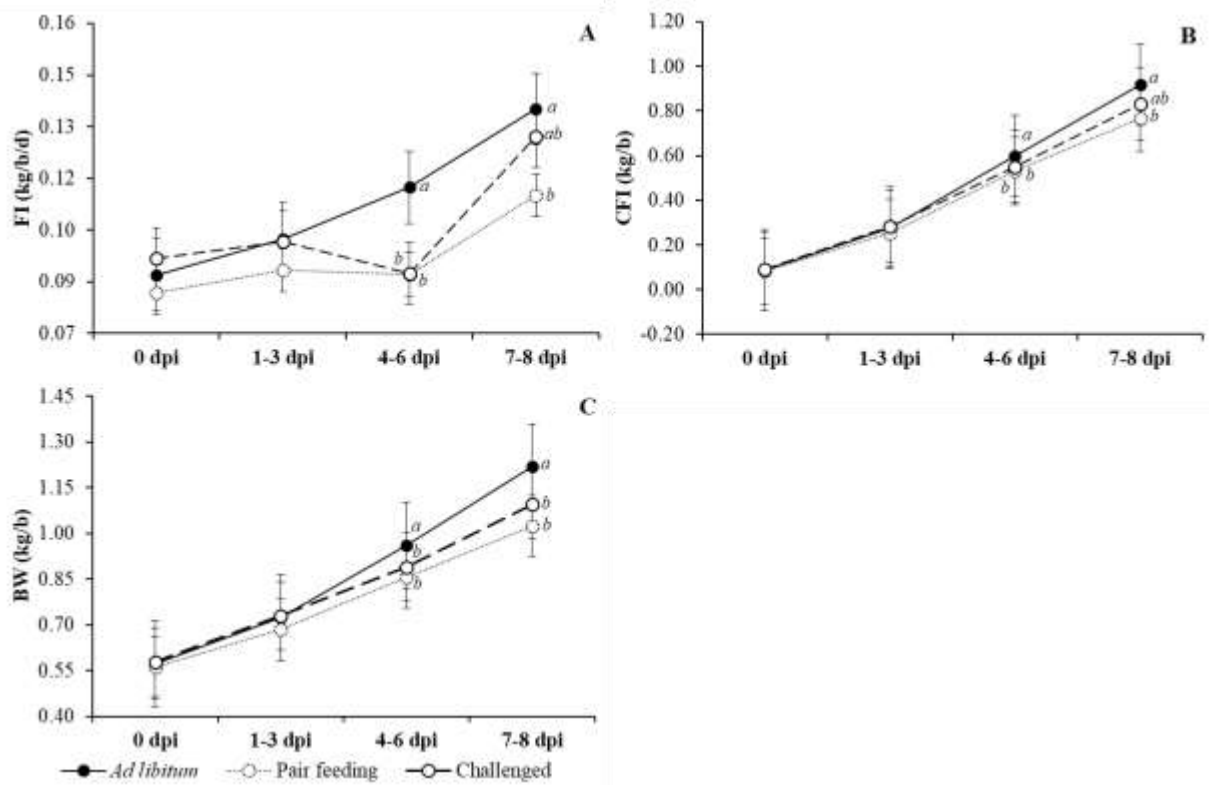


Figure 2. Daily variation of feed intake (A), cumulative feed intake (B), and body weight (C), of broiler chickens subjected to non-challenge *ad libitum* (●), challenged by *E. maxima* and *C. perfringens* (○) and pair feeding to challenged treatment (◌). The different letters at the same period (*a* and *b*) represent statistical differences ($P < 0.05$) by the Tukey test. dpi= days post-infection.

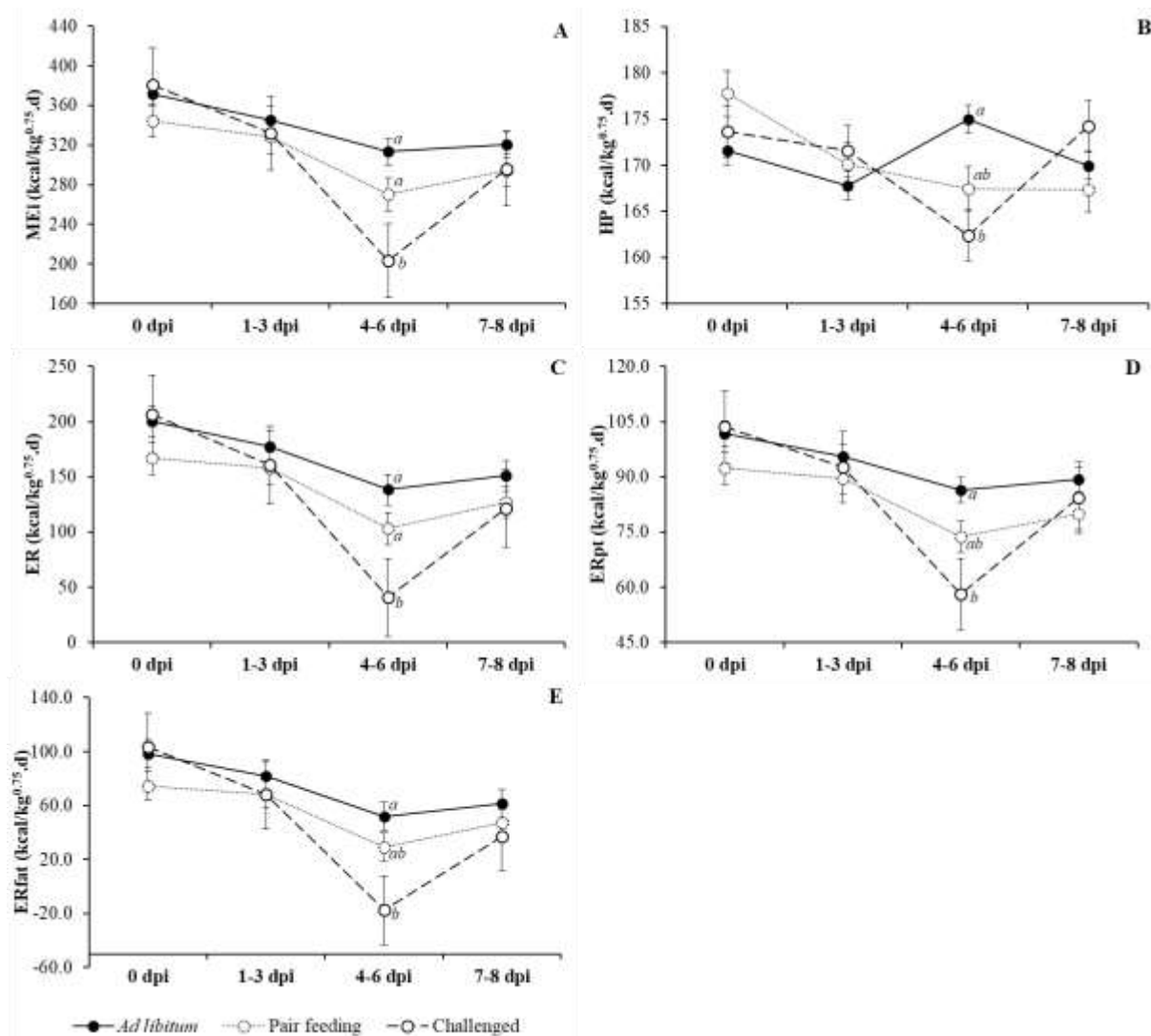


Figure 3. Metabolizable energy intake (A), heat production (B), energy retained (C), energy retained as protein (D) and fat (E) of broiler chickens subjected to the treatments non-challenge *ad libitum* (●), challenged by *E. maxima* and *C. perfringens* (○) and pair feeding to challenged treatment (◊). The different letters at the same period (a and b) represent statistical differences ($P < 0.05$) by the Tukey test. dpi= days post-infection.

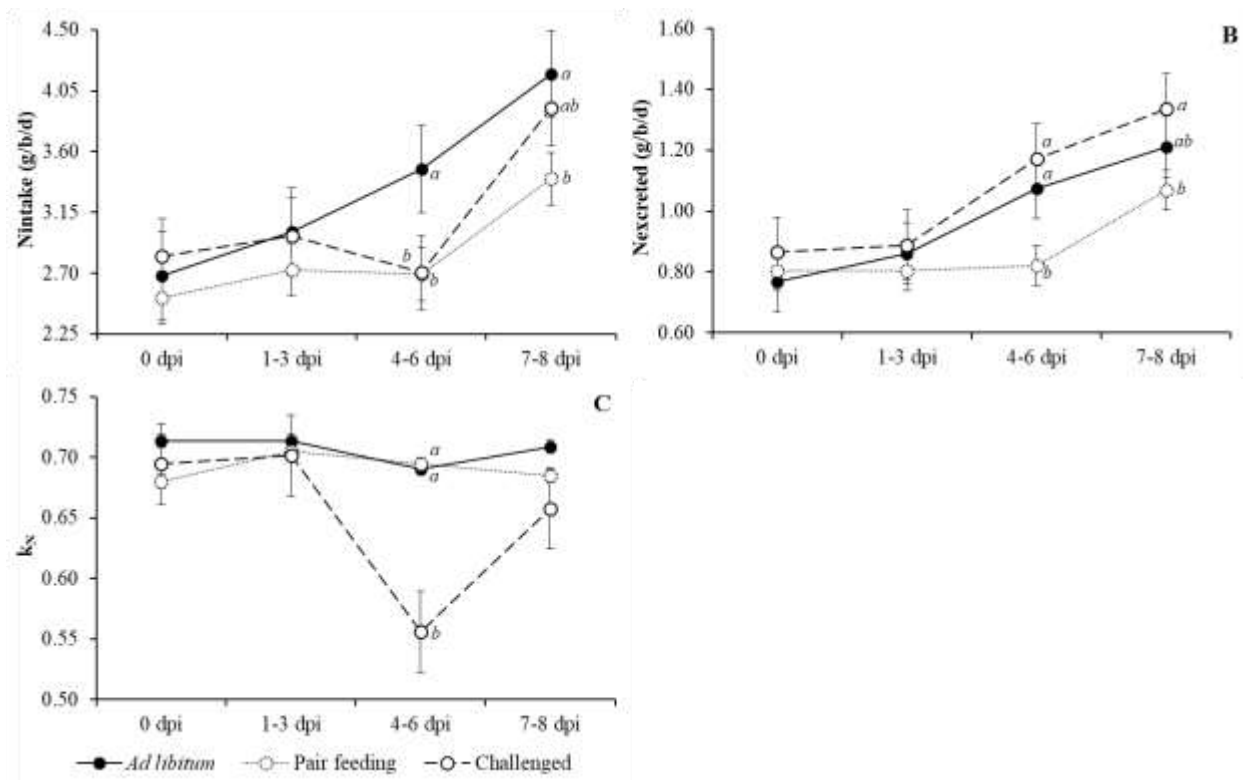


Figure 4. Daily variation on the nitrogen intake (A), nitrogen excreted (B), and efficiency of nitrogen utilization (K_N) (C) subject to the treatments non-challenged *ad libitum* (●), challenged by *E. maxima* and *C. perfringens* (○) and pair feeding to challenged treatment (○). The different letters at the same period (a and b) represent statistical differences ($P < 0.05$) by the Tukey test. dpi= days post-infection.

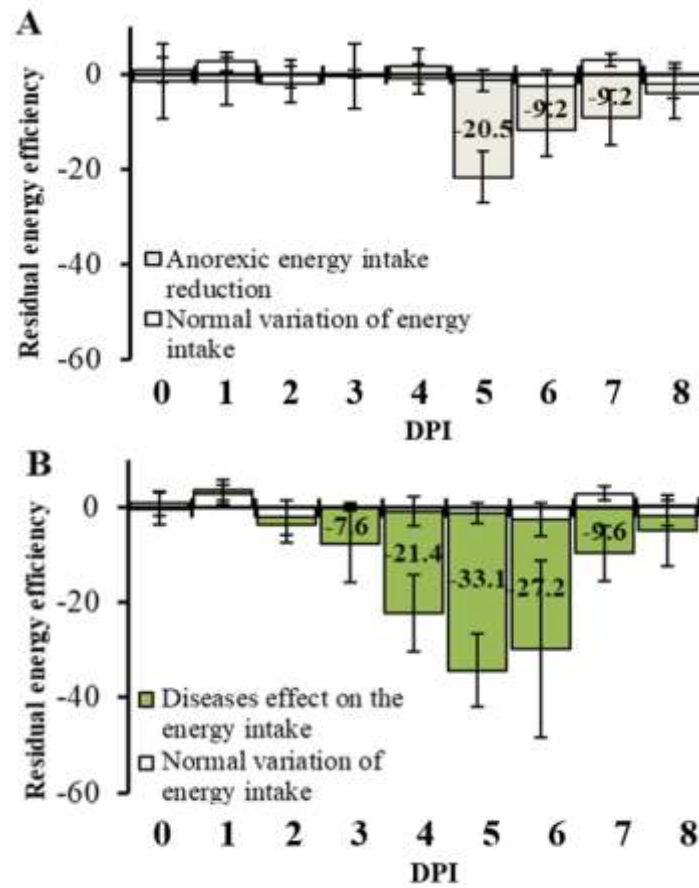


Figure 5. Residual energy efficiency calculated as the relative difference between the observed and predicted energy intake from the *ad libitum* birds to describe the anorexic effect on the energy intake reduction (A) and the effect of the diseases (B).

Table 1. Diet and nutritional composition

Ingredients	%
Corn (7.88%)	60.75
Soybean Meal (45%)	30.09
Meat and bone Meal (45%)	2.68
Oil	3.30
Limestone	0.49
Salt	0.46
Methionine hydroxy analogue (MHA-Ca 84%)	0.48
L-Lysine 78%	0.33
L-Threonine 98%	0.12
Premix Vit + Min ¹	0.20
Choline Chloride 60%	0.10
Inert ²	1.00
Calculated nutritional composition	
Crude protein (%)	20.18
Metabolizable energy (kcal/kg)	3107
Calcium (%)	0.81
Available phosphorus (%)	0.39
SID lysine (%)	1.18
SID methionine + cysteine (%)	0.93
SID threonine (%)	0.78

¹ Content/kg of feed: vitamin A 9,700 IU (all-trans retinol); vitamin D3 2,700 UI (cholecalciferol), vitamin E 17 UI (cholecalciferol); vitamin K3 2.79 mg; vitamin B1 2.00 mg; vitamin B2 5.50 mg; pantothenic acid 10.6 mg; vitamin B6 3.05 mg; vitamin B12 15.0 mcg; niacin 0.039 g; folic acid 1.00 mg; biotin 0.0083 mg; choline chloride 0.150 g; iron 0.044 g; copper 9.00 mg; manganese 0.050 g; zinc 0.050 g; iodine 1.00 mg; selenium 0.250 mg.

²Sand was used as the inert.

**CAPÍTULO 3 - Metabolomic evaluation associated with energy expenditure
and demand for internal body temperature change in
response to lipopolysaccharide challenged broilers**

Este capítulo é apresentado de acordo com as normas da revista "Poultry Science".

LIPOPOLYSACCHARIDE CHALLENGE IN BROILERS CHICKENS

Energy expenditure and thermal variation in response to lipopolysaccharide challenge in broilers: an metabolomic approach

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ABSTRACT

Health challenges in broiler chickens cause imbalances in metabolic, physiological, and immunological functions, directly impacting production and performance. This study aimed to elucidate the relationship between body temperature and energy metabolism in birds subjected to two degrees of lipopolysaccharide (**LPS**) challenge, assessing energy expenditure and exploring related metabolites. A total of 21 one day of age male Cobb 500 broilers were raised in cages until 18d. At 19d, they were assigned to three treatments: Control non-challenged birds (**CON**), Challenged birds with 10 µg *Escherichia coli* LPS/kg BW (**10LPS**), and Challenged birds with 100 µg/kg BW (**100LPS**). At 21d, the corresponding LPS dose was administered i.m. (1ml/kg BW), and the treatment **CON** birds received saline. BW, gas exchange, and core body temperature were recorded for 23h. Feed was removed to measure fasting heat production. At 22 d, blood and liver samples were collected for metabolomic analysis. There was no difference on BW among treatments ($P>0.05$); however, the **100LPS** treatment showed lower heat production (**HP**) ($P<0.05$), but with no difference compared to **10LPS** treatment ($P>0.05$). The respiratory quotient (**RQ**) and body temperature were higher in **10LPS** ($P<0.05$) when compared **CON** and **100LPS**. The **10LPS** group exhibited metabolic changes compared to the **CON** group, and the **100LPS** group presented hypothermia and lower RQ. A total of 50 and 51 metabolites were identified and quantified in the ^1H -NMR spectra of serum and liver, respectively, including amino acids, organic acids, nucleic acids, vitamins, cellular antioxidants, and others. Comprehensive analysis of the metabolomic data showed that 17 and 27 pathways were significant for serum and liver, respectively, emphasizing their use in attempting to control immunological challenge and minimize damage. By understanding how different degrees of LPS challenge affect energy metabolism, body temperature, and specific metabolomic pathways, diets can be formulated to better support energy metabolism and immune function under health challenge conditions. Additionally, the identified metabolic markers can be used to more accurately monitor the health and immune status of broilers, allowing for earlier interventions and more targeted treatments.

Key words: Core body temperature; Heat production; Lipopolysaccharide; Metabolomic; Respiratory quotient

INTRODUCTION

The production of broiler chickens stands out as the main activity of poultry farming, providing a large amount of high-quality animal protein for human consumption (Zampiga et al., 2021). However, the intensive production system of modern broiler farming exposes birds to various stressor factors that can compromise their health, welfare, and performance (Wilcox et al., 2024). Among the main stressors, birds can be continuously exposed to pathogenic challenges, which stimulate the immune system and induce several physiological and metabolic changes (Reisinger et al., 2024).

Lipopolysaccharide (**LPS**) is an endotoxin present in the membrane of Gram-negative bacteria, and is commonly used as a model to induce acute immune response, upon recognition by the non-specific or innate immune system (Gray et al., 2013; Maldonado et al., 2016). Mild infections or low doses of LPS typically activate immune defense mechanism, often accompanied by fever, which incurs significant metabolic costs due to increase in body temperature (Gray et al., 2013; Dantonio et al., 2016). In contrast, severe infections, high doses of LPS, or challenging conditions such as nutritional deficiencies, cold stress, or exposure to toxins, may trigger an energy conserving response characterized by regulated hypothermia (Steiner and Romanovsky, 2019; Amaral-Silva et al., 2020; 2021; 2022). In addition, exposure to the endotoxin can lead to detrimental effects, including systemic inflammation, metabolic dysregulation, and alterations in core body temperature regulation (Page et al., 2022).

Metabolic heat production, predominantly derived from nutrient oxidation or endogenous tissue catabolism, is the principal source of energy to regulate and maintain core body temperature. Therefore, deviations from normothermia, underlying physiological disturbances or environmental stressors require an increase or decrease of metabolic heat production (Ruuskanen et al. 2021). Improving knowledge of how health

challenges affect chickens' metabolism could provide a more comprehensive view of the system-wide metabolism and mechanisms of these event, thus potentially leading to improved prophylaxis or treatments.

In this line of reasoning, metabolomics might be used to better understand animal's metabolism and enhances our understanding of the mechanisms involved in the energy metabolism changes and the physiological adaptation of birds during infection. This allows for the evaluation of how changes in diet, environment, or exposure to pathogens may drive the metabolism, altering the metabolites profile and metabolic pathways. This provides a model for understanding the host's physiological and metabolic transitions (Afrouziyeh et al., 2022). Furthermore, it aids in discovering or interpreting potential biological mechanisms and control strategies (Mozzi et al., 2013).

Under optimal sanitary conditions, the feed supplied is the main source of energy for the bird to produce heat and grow the animals. However, during immune stress conditions, energy metabolism is adapted to face the challenge, directing resources to produce molecules (pro-inflammatory cytokines and antibodies), and principally increasing metabolic heat production to induce either fever or regulated hypothermia. The change in energy metabolism during immune challenges in chickens are not yet fully understood (Liu et al., 2014). Despite the recognized importance of thermoregulation in poultry health and performance, limited research has explored the relationship between body temperature and heat production (**HP**) during LPS challenges in broilers. Investigating how LPS-induced immune activation influences HP dynamics and body temperature regulation in broilers is essential for understanding the mechanistic underpinnings of host-pathogen interactions and identifying potential stress biomarkers, metabolic pathway alteration, and disease susceptibility.

By integrating physiological measurements of body temperature, and HP with

metabolites analyses, we aim to unravel the complex interplay between host metabolism and thermoregulatory processes in broiler chickens facing immune stressors. As the severity and time exposed to health challenges increases, there will be a corresponding impact on the metabolic rate, supporting the hypothesis that different degrees of health challenge in broilers can affect their energy metabolism due to core body temperature regulation. Therefore, the objective of this study was to elucidate the relationship between body temperature and the energy metabolism in birds subjected to two levels of LPS challenge (low and high), thus determining the effect of the degree of challenge on energy expenditure and exploring the metabolites related with the induced infection.

MATERIALS AND METHODS

Ethical Implications

All experimental procedures of utilization and management were approved by The Ethics Committee on Animal Use of the School of Agricultural and Veterinary Sciences (FCAV), São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil, under protocol number n°.5399/20.

Animals and Experimental Design

This study was carried out at the Poultry Science Laboratory of São Paulo State University (LAVINESP), Jaboticabal – SP, Brazil. A total of 21 one day of age male Cobb 500 broiler chickens were distributed into three groups of seven birds each. The birds were allocated in cages (0.80 m × 0.70 m × 0.40 m), up to 19d of age. The assays were conducted under thermoneutral conditions according to the recommendation of the genetic guidelines (Cobb Vantress, 2022).

At 19d of age, the birds were randomly distributed to the experimental treatments, considering the same average of BW of 1.027 ± 0.05 kg between treatments. The

experimental treatments were: control non-challenged birds (**CON**), challenged birds with 10 µg of LPS/kg BW (**10LPS**), and 100 µg of LPS/kg BW (**100LPS**). Birds in the stress groups were injected with LPS from *Escherichia coli* serotype O127:B8 (Sigma-Aldrich, Brazil) to induce the immune stress model. Each treatment consists of seven replications of individual observation.

Animal Management and Challenge Protocol

Throughout the pre-experimental period, the birds were housed in climate-controlled conditions, ensuring *ad libitum* access to both water and feed. The lighting program used 23h of light for the entire experimental period (21d). At 19d of age, the birds were transferred to individual open-circuit respirometry chambers for two days of acclimatization period. The measurements and data collection were performed at 21d of age. The outlined protocol and data collection procedure are detailed in Figure 1.

At the start of the experimental period, during the morning (09:00h) the birds of the challenged treatments (**10LPS** and **100LPS**) were injected via i.m. application (1ml/kg BW) in the pectoralis major muscle with their respective LPS doses. Birds in the **CON** treatments received 1 ml of saline solution/kg BW to standardize the handling stress. At the same time, the feed was withdrawn to measure HP under fasting state and water was maintained *ad libitum*.

The BW (kg) was collected at the start and final of the experimental period (21d). The gas exchanges parameters (oxygen consumption – $\dot{V}O_2$ and carbon dioxide production – $\dot{V}CO_2$) and core body temperature were continuous and simultaneously recorded by 23h.

Indirect Calorimetry

The indirect calorimetry system consisted of two individual open-circuit chambers (0.50 m × 0.40 m × 0.50 m). The schematic description of the system of

chambers, flowmeters and analyzers and the calibration protocol are in supplementary material (Suppl. 1). Each chamber had an internal gas mixture fan and was coupled to the Sable System Classic Line components (Sable System, NV, USA) for air flow pumping, sampling, and analysis. Environmental air was drawn into the chamber using negative pressure generated by the flow kit pump (FK-100, Sable System, NV, USA). A sample or around 160 mL/min of air was automatically collected for analysis. The sequence of sampled air analyses was the water vapor pressure (RH, Sable System, NV, USA) measurement. The air sample was then dried using CaSO_3 to analyze the O_2 and CO_2 concentrations in dry air to express the volumetric concentration for a gas volume corrected to standard barometric pressure. The gas concentrations for O_2 and CO_2 were determined using a paramagnetic (PA, Sable System, NV, USA) and an infrared (CA, Sable System, NV, USA) analyzers, respectively. Data recording of gas exchange concentration and flow were programmed to register each data per second. To facilitate the calculations of HP, individual data were computed and averaged per minute. The volume (L/min) of $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$, respiratory quotient (**RQ**), and HP data were calculated according described by Lighton (2018) for negative-pressure open-circuit system chambers.

The in-going airflow (F_{in} , L/min) was calculated as follows:

$$F_{in} = (F_{out} * \frac{(100 - [\text{O}_2]_{out} - [\text{CO}_2]_{out})}{(100 - [\text{O}_2]_{in} - [\text{CO}_2]_{in})}) \times 1.440$$

The $\dot{V}\text{O}_2$ (L/d) and $\dot{V}\text{CO}_2$ (L/d) were obtained as:

$$\begin{aligned}\dot{V}\text{O}_2 &= F_{in} \times [\text{O}_2]_{in} - F_{out} \times [\text{O}_2]_{out} \\ \dot{V}\text{CO}_2 &= F_{out} \times [\text{CO}_2]_{out} - F_{in} \times [\text{O}_2]_{in}\end{aligned}$$

Where F_{out} is the outgoing airflow, $[\text{O}_2]_{in}$, and $[\text{CO}_2]_{in}$ are the atmospheric gas concentrations. The HP (kcal/d) was calculated by the Brouwer equations (1965) and expressed per unit of metabolic BW ($\text{BW}^{0.75}$):

$$HP \text{ (kcal)} = 3.86 \times \dot{V}O_2 + 1.20 \times \dot{V}CO_2$$

Therefore, the RQ was calculated as the $\dot{V}CO_2/\dot{V}O_2$ ratio.

Core Body Temperature

A temperature datalogger DS1221H-F5# (iButtonLink, LLC, Whitewater, WI, USA) was used to record core body temperature. The device is a small size (17.35 mm diameter $\times$ 5.89 mm height), with high sensitivity and resolution for temperature. The sensor has a temperature range from 15°C to 46°C, with an accuracy of $\pm 1^\circ\text{C}$ and a resolution of 0.125°C.

The sensor was surgically implanted into the coelomic cavity at 14d of age, and an interval of 7d was considered for post-surgical recovery. The surgical procedure for sensor implantation was performed in anesthetized birds using isoflurane (3% for induction, 1% for maintenance) diluted in 100% oxygen through an open-circuit facial mask connected to a specific vaporizer (VetCase Brasmed, São Paulo, Brazil). Once the withdrawal reflex was lost, an incision was made in the coelomic cavity using a scalpel, and the sensor was inserted. The incision site was closed using internal and external sutures. The entire procedure took approximately 20 min, and the animals were observed until they fully recovered from anesthesia. After complete recovery, the chicks were relocated to a clean cage. To prevent infection and pain, the chicks were treated with antibiotic (enrofloxacin, 10 mg/kg⁻¹) and non-steroidal anti-inflammatory (flunixin meglumine, 2.5 mg/kg⁻¹) agents during the surgery and for two more days. The sensors were programmed to start recording the core body temperature during the experimental period from 14 to 22d, with a frequency of 12 data recording per hour (1 data of temperature/5 min).

Metabolomics Analysis

Sample Preparation: At 22d of age, finalizing the indirect calorimetry trial, a

blood sample (10 mL) were collected from a punctured wing vein and placed in dry 10 mL tubes (BD Vacutainer, São Paulo, SP, Brazil). The blood samples were centrifuged for 15 min at $2,000 \times g$ at 4°C ; the serum was collected and stored at -80°C . After that, the birds were euthanized by cervical dislocation to liver tissue sample collection. A sample (around 1 g) of the right lobe of the liver was collected, allocated in Eppendorf tubes, rapidly frozen in liquid nitrogen, and subsequently stored at -80°C . Both samples of plasma and liver were conducted for further metabolomic profile analysis.

Extraction of Polar Metabolites from Liver and Serum: The liver samples were individually transferred to a 2 mL Eppendorf tube, and 400 μL of chilled methanol and 285 μL of Milli-Q water were added. The sample was then disintegrated and homogenized (ULTRA-TURRAX® T25 digital, IKA, Campinas, SP, Brazil), followed by the addition of 400 μL of ice-cold chloroform and vortexed for 1 min, as previously described by Cônsolo et al. (2021). The samples were left on ice for 15 min and then centrifuged for 10 min at $12,700 \times g$ at 4°C to remove precipitated protein. The supernatants were carefully transferred to a new tube and storage at -80°C until nuclear magnetic resonance (^1H -NMR) spectrometry analysis.

For the extraction of serum metabolites, the preparation of 3 kDa filters was performed as previously described by Matias et al. (2024). 500 μL of Milli-Q water was transferred to the filter, which was then centrifuged at $10,000 \times g$ at 4°C for 5 min. This process was repeated five times to guarantee the sample's quality. The samples were initially thawed at room temperature for 30 min, and then 500 μL of the sample was transferred to the filter and centrifuged at $10,000 \times g$ at 4°C for 30 min. The filtered was storage at -80°C until ^1H -NMR analysis.

Sample preparation for Nuclear Magnetic Resonance measurements: The analyses were performed at EMBRAPA Instrumentação (São Carlos, São Paulo State).

The serum and liver extract were dried, and subsequently resolubilized in 550 μL of phosphate buffer (D_2O -based PBS; 100 mM; $\text{pD} = 7.4$) containing 0.5 mM of sodium 3-(Trimethylsilyl)-1-propanesulfonic acid- d_6 (DSS- d_6 , from Cambridge Isotopes, Leicestershire, UK) used as NMR internal standard. Posteriorly, a volume of 600 μL of each sample was transferred to a standard 5 mm NMR tube for NMR measurements.

Nuclear Magnetic Resonance Spectroscopy: One Dimensional (1D) NMR Spectroscopy experiments were performed on a Bruker Avance III 14.1 T spectrometer (Bruker Corporation, Karlsruhe, Baden-Württemberg, Germany) fitted with a 5 mm Broadband Observe (BBO) probe with ATMA® (Automatic Tunning Matching Adjustment), a z-field gradient, BCU-I variable temperature unit, field gradient generator unit, and a Sample-Xpress™ automatic sample changer. Standard ^1H spectra were acquired at 298.15 K using a NOESY-1D pulse sequence (named noesypr1d in the TopSpin Bruker software) with water signal suppression by irradiation at 2824.27 Hz (O1). The acquisition parameters were as follows: number scans (ns) of 256, spectral width (sw) of 12019 Hz (20.0276 ppm), 90° pulse (P1) of 12.9 μs , acquisition time (aq) of 2.72 s, relaxation delay (d1) of 4s, data points (TD) of 64 K, mixing time (d8) of 5 ms, and dummy scans (ds) of 4.

Spectral Processing and Metabolite Quantification: Proton NMR spectra were processed using Chenomx NMR Suite Professional 7.7 software (Chenomx Inc., Edmonton, Canada): phasing and baseline correction were performed. The spectra were referenced to the DSS methyl peak at 0.00 ppm. The same peak was also used as a chemical shift indicator, that is as an internal standard for quantitation.

The metabolites were quantified in the 1D ^1H -NMR spectra of serum, and liver, using the Profiler module in the Chenomx NMR Suite Professional software with an integrated 1D spectral library. Quantification was performed by comparing the peak areas

of the selected metabolite with the area under the methyl peak of DSS, which corresponded to a known concentration of 0.5 mM in each sample. The resulting metabolite concentration table was exported to Excel, where sample identifiers were added.

Statistical analysis

Previous performance data of initial, final and average of BW were individually organized in a spreadsheet, and after that one-way variance analysis (ANOVA) were performed, and mean comparisons were conducted using the Tukey Test. The variables of HP, core body temperature, and RQ were subjected to non-linear regression to describe their profile as functions of time (t). This procedure was conducted using SAS statistical software through the PROC NLIN procedure.

The model fit was as follows:

$$f(t) = A * (1 - e^{(-k_1 \times (t-t_0))}) \times e^{(-k_2 \times (t-t_1))} + C$$

Where:

$f(t)$: Represents the response variable (e.g. core body temperature, HP or RQ) as a function of time

t : Time in hours.

A : Represents the amplitude of change in the response variable. This parameter determines the maximum increase in the response variable from its baseline (C).

k_1 : The rate constant for the initial increase phase. Controls how quickly the response variable initially rises from the baseline (C).

k_2 : The rate constant for the decay phase. Indicates the rate at which the response variable decays after peaking.

t_0 : The time shift parameter for the initial increase phase.

t_1 : The time shift parameter for the decay phase.

C: The asymptotic value or minimum response variable at $t=\infty$. Represents the baseline or minimum value of the response variable after all dynamic changes have occurred.

Graphical representation of the theoretical model and the detailed description of the parameters are shown in the Figure 2. The parameters obtained for each treatment in the HP, RQ and core body temperature description were compared by Bonferroni arrangement of a t-test considering 95% of confidence intervals.

The metabolomic data were analyzed using MetaboAnalyst 6.0 (<http://www.metaboanalyst.ca/>). The metabolite concentration table was uploaded to MetaboAnalyst, and the data were log transformed and Pareto scaled before analysis. Supervised partial least squares-discriminant analysis (**PLS-DA**) was performed. Cross validation was performed for PLS-DA analysis using the method leave-one-out cross-validation and the performance measure accuracy. Further, to better explore metabolites differences between groups, the volcano plot analysis was performed on the pairwise comparisons **CON** vs **10LPS**, **CON** vs **100LPS**, and **10LPS** vs **100LPS**. Pathway analyses were conducted using metabolite data sets for each group, using the *Gallus gallus* library in MetaboAnalyst, and based on the exploratory nature of this study, we included pathways with a raw P value of < 0.1 as being of high pathway impact.

RESULTS

Body Weight Variations

As expected, there were no statistical difference ($P>0.05$) was observed in the initial and final BW, which consequently did not influence the variable and average BW ($P>0.05$), due to the selection and distribution at 19d of age ensuring the same average BW between treatments.

Energy Metabolism and Core Body Temperature

The description of the estimated parameters of HP, RQ and core body temperature, subjected to non-linear regression fit for each experimental treatments are shown in Table 1 and the graphical description of observed and predicted values are shown in Figure 3.

Heat Production

Overall, the chickens showed different HP behaviors between the **CON** and the challenged groups as shown in the Figure 3A, evidencing a difference on the dynamic of metabolic rate after infection. The challenged groups demonstrate an increase in HP after inoculation, although none of the treatments significantly affected in the time to the initial increase (t_0) and decay (t_1) phases (Table 1). The peak of HP was higher in the **10LPS** treatment (142 kcal/kg^{0.75}) compared to the **100LPS** treatment (130 kcal/kg^{0.75}). The increased HP is attributed to variation in the amplitude of the response, represented by the parameter A , with the **100LPS** group showing a higher $A=26.6$ compared to the **10LPS** group reporting the A of 15.5 ($P<0.05$). Although the rate of increase in HP from the start of the experiment to t_0 , represented by the parameter k_1 , was higher in the **CON** group, no significant difference was observed in the response curve. Notably, the rate of increase (k_1) was same ($P>0.05$) between the **100LPS** group ($k_1=0.002$) and **10LPS** group ($k_1=0.003$).

The **CON** treatment exhibited a reduction in HP shortly after the start of the experimental period, which was an expected behavior consistent with a post-absorptive state under fasting condition. However, the **CON** group displayed only a single phase of HP decline, differing from the challenged groups, which exhibited an initial increase phase (k_1) until reaching peak values during the time shift to the t_0 . The rate of HP decrease (k_2) post-peak was significantly lower ($P<0.05$) in the **CON** group ($k_2=0.147$)

compared to the challenged groups, which were $k_2=0.261$ for **10LPS** and $k_2=0.247$ for **100LPS**.

Furthermore, there were significant differences ($P<0.05$) in the HP plateau between treatments, which represents the minimum energy expenditure under fasting conditions, considered the FHP of the birds. The **100LPS** treatment presented the lowest plateau value ($110 \text{ kcal/kg}^{0.75}$), statistically different ($P<0.05$) from the **CON** group ($118 \text{ kcal/kg}^{0.75}$) which presented the highest value. The **10LPS** treatment ($114 \text{ kcal/kg}^{0.75}$) was statistically similar to both the **CON** and **100LPS** groups ($P>0.05$).

Respiratory Quotient

The RQ exhibited different patterns between the **CON** and challenged groups (Figure 3B). The increase rate for the initial increase phase (k_1) was significantly higher ($P<0.05$) in the **CON** and **10LPS** treatments compared to the **100LPS** treatment (Table 1). The start increasing time during the initial increase phase (t_0) was not significant in all treatments, exhibiting a rapid decline after reach the peak. Consequently, the peak value reflects the maximum RQ at time zero, and it was higher for the **CON** treatment (RQ for $t_0=0.997$) close to one, indicating an RQ consistent with an animal under feeding state. This was similar to the **10LPS** treatment (RQ for $t_0=0.972$) and higher than the **100LPS** treatment (RQ for $t_0=0.917$). This difference in peak RQ is mirrored in the amplitude parameter (A), where the **CON** treatment ($A=0.328$) was statistically higher ($P<0.05$) than the **100LPS** treatment ($A=0.208$) and similar to the **10LPS** treatment ($A=0.260$).

Since the **CON** group started with a higher RQ, these birds exhibited a lower decreasing rate ($k_2=0.126$) ($P<0.05$) than the **10LPS** ($k_2=0.227$) and **100LPS** ($k_2=0.223$) treatments. Additionally, the time after reach the peak and pass for the decay phase (t_1) was similar across all treatments, with this change occurring at 2.03 h. At the RQ plateau (C), the **CON** group reached the RQ-plateau=0.713, statistically equal ($P>0.05$) to the

100LPS treatment, which showed the RQ-plateau=0.737, while the **10LPS** treatment was significantly different from both ($P<0.05$), presenting a higher RQ-plateau=0.793.

Core Body Temperature

Regarding core body temperature, the **CON** group presented different behaviors compared to the challenged groups (Figure 3C), similar to the HP and RQ. The increasing rate (k_1) from t_0 to t_1 , was statistically different for all treatments ($P<0.05$), the **CON** treatment presented the highest increasing rate up to reach the peak at $k_1=0.327$, followed by the **10LPS** treatment with $k_1=0.048$, and the lowest value for the **100LPS** treatment that presented a k_1 of 0.015 (Table 1). In this sense, the amplitude between the base line and the peak, also varied significantly ($P<0.05$) among treatments, with the **CON** group exhibiting the lowest $A=0.889$, followed by the **10LPS** group evidencing the $A=3.52$, and with the highest $A=6.09$ for the **100LPS** group.

In terms of the time where start the increasing of body temperature (t_0), it not was possible to estimate for the **CON** treatment, indicating that body temperature did not evidence an increasing phase. On the other hand, the challenged treatments reached a plateau at $t_0=0.752$ h and $t_0=0.446$ h for the **10LPS** and **100LPS** groups, respectively. The challenged birds showed a significant increase in body temperature, as indicated by peak values of 41.06°C and 40.95°C for the **10LPS** and **100LPS** infection doses, respectively. The predicted peak body temperature for the **CON** group was 40.44°C, reflecting standard conditions with minimal variation.

Following the peak, the temperature subsequently decreased, represented by the rate of the decay phase (k_2). The **10LPS** and **100LPS** groups presented a high rate ($k_2=0.226$, and $k_2=0.290$, respectively), while the **CON** group had a significantly ($P<0.05$) lower rate of ($k_2=0.147$). Although both challenged treatments presented reduction phases, they presented significantly different ($P<0.05$) plateau (C) values. The **100LPS** group had a

more pronounced reduction, reaching a plateau of 39.8°C, compared to the **10LPS** group (40.0°C) and was statistically equal ($P>0.05$) to the **CON** group (39.9°C).

Metabolomics analysis

A total of 50 and 51 metabolites were identified and quantified in the 1D ^1H -NMR spectra of serum and liver, respectively, including non-essential amino acids, essential amino acids, small peptides, amines, organic acids, nucleic acids, sugars, vitamins, and cellular antioxidants.

Serum

The PLS-DA was used to visualize the differences in metabolite profiles between the treatments ($R^2 = 0.89$; Figure 4). The score plot (Figure 4) shows ellipsoids in different spectra, indicating a separation between the **CON** group, and the challenged groups (**10LPS** and **100LPS**), suggesting that their metabolic profiles are different. However, the **10LPS** group overlaps with the **100LPS** group, indicating that the metabolic profiles share similarities. To further explore metabolic differences between pairwise comparisons, a volcano plot was performed.

Metabolite Profiles: The Volcano Plot was performed for all comparisons **CON** vs **10LPS**, **CON** vs **100LPS**, and **10LPS** vs **100LPS** are shown in Figures 5A, 5B and 5C. Animals from **CON** group presented greater amounts of trans-4-Hydroxy-proline, arabinose, methylsuccinate, threonine, arginine, and succinate compared to animals from **10LPS** (Figure 5A). When compared to **100LPS**, animals from **CON** group also presented greater concentration of 2-Hydroxy-Butyrate, glutamate, and arabinose alanine, cystine, leucine, threonine, succinate, 3-Hydroxybutyrate, valine, and proline (Figure 5B) on their serum. But when the pairwise comparison **10LPS** and **100LPS** (Figure 5C) was performed, alanine was the only metabolite responsible for groups differentiation, with its concentration in **10LPS** being double that in **100LPS**.

Enrichment Analysis: The results of enrichment analysis of serum metabolites affected by LPS doses in broiler chickens are summarized in Table 2. In the comparison between **CON** treatment vs. **10LPS**, six pathways were significantly enriched ($p < 0.05$), namely: Threonine and 2-Oxobutanoate Degradation, Arginine and Proline Metabolism, Oxidation of Branched Chain Fatty Acids, Mitochondrial Electron Transport Chain, Phytanic Acid Peroxisomal Oxidation, and Propanoate Metabolism. In the comparison between **CON** treatment vs. **100LPS**, nine pathways were significantly enriched ($p < 0.05$), namely: Propanoate Metabolism, Cysteine Metabolism, Arachidonic Acid Metabolism, Valine, Leucine and Isoleucine Degradation, Lysine Degradation, Ketone Body Metabolism, Tryptophan Metabolism, Folate Metabolism, and Glucose-Alanine Cycle. When comparing doses **10LPS** vs. **100LPS**, two pathways tended to be enriched, namely: Alanine Metabolism, and Histidine Metabolism.

Liver

The first two components of PLS-DA for liver samples explain 51.3% of the data variance, the model is valid according to the cross-validation test results ($R^2 = 0.88$). The score plot (Figure 6) shows ellipsoids in the same spectra, indicating that the metabolic profiles share similarities between the control group (**CON**) and the challenged groups (**10LPS** and **100LPS**), suggesting that their metabolic profiles are similar.

Metabolite Profiles: Cholate was the most important metabolite for discriminating between **CON** vs. **10LPS** and **CON** vs. **100LPS** (Figure 7A and B), with its concentration in **CON** being lower than **10LPS** and **100LPS**. Choline, glycine, phenylalanine, glutamine, dimethylamine, sn-Glycero-3-Phosphocholine, and ascorbate were also important for discriminating between **CON** vs **10LPS** (Figure 7A), with the last two metabolites presented lower concentration in **10LPS** group compared to **CON**. On the pairwise comparison **CON** vs. **100LPS**, betaine, succinate, guanidoacetate, and

ascorbate (Figure 7B) presented greater concentration on **CON** group. Tryptophan was the most important metabolite for discriminating between the challenge doses **10LPS** and **100LPS** (Figure 7C), presenting greater concentration on animals challenged by **100LPS**, other metabolites, such as 3-Hydroxybutirate, glycine, glutamate, serine, and succinate with greater concentration for **10LPS** animals.

Enrichment Analysis: The results of the enrichment analysis of metabolites in the liver affected by LPS doses in broiler chickens are summarized in Table 3. In the comparison between the **CON** treatment vs. **10LPS**, twelve pathways were significantly enriched ($p < 0.05$), namely: Bile Acid Biosynthesis, Methionine Metabolism, Porphyrin Metabolism, Phosphatidylethanolamine Biosynthesis, Phospholipid Biosynthesis, Phosphatidycholine Biosynthesis, Carnitine Synthesis, Betaine Metabolism, Glutathione Metabolism, Catecholamine Biosynthesis, Alanine Metabolism, and Retinol Metabolism. In the comparison between **CON** treatment vs. **100LPS**, nine pathways were significantly enriched ($p < 0.05$), namely: Ketone Body Metabolism, Oxidation of Branched Chain Fatty Acids, Phytanic Acid Peroxisomal Oxidation, Butyrate Metabolism, Carnitine Synthesis, Betaine Metabolism, Bile Acid Biosynthesis, Mitochondrial Electron Transport Chain, and Methionine Metabolism. When comparing doses **10LPS** vs. **100LPS**, nine pathways were significantly enriched ($p < 0.05$), namely: Ketone Body Metabolism, Glycine and Serine Metabolism, Fatty Acid Biosynthesis, Alanine Metabolism, Methionine Metabolism, and Ammonia Recycling.

DISCUSSION

There are significant nutritional costs associated with immune activation in response to infection, including decreased feed intake, fever, and redistribution of nutrients away from anabolic processes. Although the exact balances of nutrients and

energy required for a robust immune response are not fully understood, it is clearly that immune system demands a continuous supply of nutrients, which increases during pathogen challenge (Humphrey et al., 2006). Thus, these nutritional requirements to maintain a competent immune system and to mount an immune response likely result in trade-offs with competing processes such as growth (Lee, 2006), potentially affecting the vigor with which an organism defends itself. The costs of an immune response can be expressed in terms of the amounts of energy diverted to the immune system, reduction in tissue deposition, and impairment of vital functions.

The interactions between nutrition and immunity are complex and have profound implications for animal growth. A decrease in BW is expected to be a consequence of an acute inflammatory response due to a general catabolic state and reduced feed intake (Johnson, 1997; Camargos et al., unpublished data). However, the experimental protocol of our study was conducted in birds under fasting conditions, the influence of feed intake was disregarded, and the chickens in the three treatments showed similar BW after 24h post-application. Wu et al. (2013) similarly found that the body weight gain of chickens administered with LPS was not significantly different from those that received only saline solution, evidencing that bird exposed by short period to this challenge model is not sufficient to evidenced variations on the BW, even the dynamic of endogenous sources can be changed during the challenged period.

It is well established that disease challenge has significant effects on metabolic, endocrine system and enzymatic activity (Julian, 2005), all of which negatively affect the bird performance. In terms of the metabolic adaptation under challenge conditions, it can be evaluated by metabolic rate measurement – expressed by HP. Our study demonstrated that the HP patterns of the challenged groups differed significantly from those of the control birds, reflecting distinct dynamics in energy utilization in response to the LPS

challenge. Notably, the HP patterns were mirrored within the challenged groups, suggesting that different LPS levels induced similar adaptive responses. Despite the rapid response of birds to LPS at both levels, they exhibited a remarkable capacity for metabolic adaptation, particularly in relation to variations in body temperature, which we hypothesize as a key factor driving these responses.

The influence of disease and sanitary conditions on HP variation has been inconsistently reported in the literature. Some studies suggest that health challenges can negatively impact HP by increasing metabolic demands (M'Sadeq et al., 2016), while others report that health challenges may not significantly affect HP but do lead to reduced energy intake and reliance on endogenous energy sources, particularly reducing energy retention in the form of fat (Camargos et al., unpublished data). However, most existing research has been conducted over extended periods, making it difficult to fully understand the immediate metabolic adaptations during acute immune challenges. Our study provides new insights into these short-term adaptations, highlighting the dynamic interplay between energy metabolism and immune response.

Our findings indicate that both HP and core body temperature followed similar patterns within the challenged groups, differing from those in the control group. Both variables exhibited a sharp increase post-inoculation, with higher LPS doses resulting in more pronounced increases. This suggests that after inoculation, the birds experienced an increase in metabolic rate, peaking around four hours post-inoculation in both challenged groups. Interestingly, body temperature increased more rapidly, reaching a plateau within one hour after infection. This suggests that the nonspecific immune response, indicated by the initial rise in body temperature, occurs more quickly than the metabolic adaptation. The delay in reaching peak HP compared to peak body temperature, especially in the higher LPS dose group, suggests a more gradual development of fever. This metabolic

cost is reflected in a lower HP plateau, indicating that the energy demand to sustain the increased body temperature was significant (Webb, 1995).

The dynamic of HP is closely linked to the oxidation rates of available substrates, which is represented by the RQ (Gerrits et al., 2015). The RQ patterns in the control group differed markedly from those in the challenged birds, showing a much faster decline, consistent with fasting conditions. The control birds reached a minimum RQ around 0.72, similar to values reported in other studies on fasting birds (Anderson and Kulp, 1922; Misson, 1974; Lopez and Leeson, 2008; Teofilo et al., 2022). This decline in RQ is directly related to the catabolism of endogenous tissue, primarily through fat oxidation, as indicated by an RQ close to 0.7 (Gerrits et al., 2015).

Interestingly, while all birds showed a similar pattern in RQ decline, the plateau RQ differed between the challenged groups. The lower LPS dose group exhibited a higher RQ plateau, suggesting that these birds prioritized readily available energy sources, such as body tissue catabolism, during the early stages of infection. As fasting continued, fat reserves diminished, requiring the activation of alternative energy pathways, such as gluconeogenesis, to sustain essential functions. In this context, ketone bodies, which serve as an alternative energy source, particularly for brain and muscle tissues, become critical (Qi et al., 2022). The presence of lower levels of 2,3-hydroxybutyrate in serum reflects its role in replenishing energy within extrahepatic tissues (Robinson and Williamson, 1980).

Moreover, the liver's response during the LPS challenge involved the activation of pathways related to ketone body and fatty acid metabolism. Lean mass catabolism was also evident, particularly through the oxidation of branched-chain amino acids like isoleucine and valine, which contributed to metabolic heat production and influenced the RQ. These amino acids, with higher RQ values close to 0.8, undergo oxidation, and their

nitrogen groups are directed toward excretion, entering the carnitine synthesis pathway. The activation of gluconeogenesis further increases acetyl-CoA synthesis, which in turn enhances butyrate synthesis and activates the mitochondrial electron transport chain, sustaining high rates of NADH and FADH production necessary for energy metabolism (Utter et al., 1964).

Comparing the challenge treatments, the lower level of challenge allowed birds to conserve some energy due to fever, leading to the catabolism of lean tissue as the primary energy source. This was evidenced by lower levels of methionine, threonine, glycine and tryptophan in the challenged groups, playing crucial roles in maintaining the REDOX system and antioxidant defenses under stress (Martínez et al., 2017; Min et al., 2017; Deng et al., 2023; Ouyang, et al., 2023). Arginine, present in the blood, was utilized primarily for nitric oxide production, an essential molecule for pathogen clearance and immune modulation (Martí i Líndez and Reith 2021). Arginine deficiency, often observed under challenging conditions, likely results from its use in immune responses and cellular repair mechanisms. During the LPS challenge, increased arginine metabolism enhances nitric oxide production, which plays a significant role in regulating body physiology and the inflammatory response, including fever induction.

Alanine also played a critical role during the immune challenge (Park et al., 2024), facilitating glucose production, detoxifying nitrogenous waste, and supporting protein synthesis. Its involvement in these processes underscores its importance in maintaining physiological balance and promoting recovery during periods of stress and infection. Alanine metabolism plays a crucial role in this process, helping to balance nitrogen and energy production during immune challenges in broilers. The breakdown of amino acids in muscle releases nitrogen, which is incorporated into alanine, transported to the liver, and converted into urea for excretion, thereby preventing toxic ammonia accumulation

and maintaining nitrogen balance during protein catabolism in immune responses (Holeček, 2024).

These findings are supported by serum metabolomic data, where lower levels of free amino acids were observed in the blood of **10LPS** animals, suggesting their use as an energy source. Additionally, the most abundant metabolite in the serum of birds in the **CON** vs. **10LPS** comparison was trans-4-hydroxy-L-proline, produced by the hydroxylation of proline (Adams and Frank, 1980). Its low concentration in the **10LPS** group may be due to its role in regulating phosphorylation, catalytic activation, and cellular signaling, contributing to cellular metabolism, growth, and responses to nutritional and physiological changes (Hu et al., 2022).

Fever, as a regulated increase in body temperature, is part of the complex physiological reaction to invading pathogens, adjusting the thermoregulatory system to retain and produce heat while minimizing heat loss (Gray et al., 2013). In our study, a febrile response preceded by hypothermia was observed, with more pronounced fever in the **10LPS** group and hypothermia in the **100LPS** group. High doses of LPS tend to induce regulated hypothermia rather than fever, with both responses considered adaptive (Romanovsky et al., 1996; Steiner and Romanovsky, 2019; Amaral-Silva et al., 2021). Hypothermia, by reducing tissue hypoxia, acts as a defensive response after sepsis or high doses of LPS administration (Liu et al., 2012). A decrease in body temperature reduces metabolic demands, potentially aiding in tissue protection during septic shock (Blair et al., 1964). Fever, however, alters glucose metabolism, with increased glycolysis providing energy for the immune response, while gluconeogenesis maintains blood glucose levels during periods of stress or fasting.

In conclusion, the findings highlight the complex trade-offs between immune system activation and energy metabolism, demonstrating distinct patterns of HP and RQ

in LPS-challenged birds. These patterns reflect a dynamic reallocation of energy resources, particularly through fat and amino acid oxidation, to support the immune response. LPS-induced febrile responses and metabolic adaptations are dose-dependent, with higher doses leading to hypothermia as an adaptive response, reducing tissue oxygen demands and metabolic rates, and lower doses trigger a febrile response, prioritizing the use of adipose and lean tissue reserves to sustain the immune system. The shift in amino acid metabolism, particularly in alanine, methionine, and arginine, underscores the role of these molecules in energy balance, immune modulation, and antioxidant defenses during stress. These findings have important implications for enhancing nutritional strategies and management practices in animal production, particularly for optimizing immune competence without compromising growth or performance.

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Table 1. Heat production (HP), respiratory quotient (RQ), and core body temperature (Tb) data of broilers submitted to LPS treatments (10 µg/kg-10LPS and 100 µg/kg-100LPS) subjected to non-linear regression

Variable	Treatment	Parameters						Peak value [†]	SEM	P-value
		A	k ₁	k ₂	t ₀	t ₁	C			
HP (kcal/kg ^{0.75} /d)	<i>Control</i>	15.8±1.50 ^b	12.2±0.118 ^a	0.147±0.001 ^b	0.010±0.001	.	118±6.87 ^a	131	10.1	<0.050
	<i>10LPS</i>	15.5±1.17 ^b	0.003±0.001 ^b	0.261±0.017 ^a	0.137±0.011	23.6±0.700	114±5.52 ^{ab}	142	8.82	<.0001
	<i>100LPS</i>	29.6±0.291 ^a	0.002±. ^b	0.247±0.068 ^a	.	21.5±0.077	110±2.08 ^b	130	11.3	<.0001
RQ	<i>Control</i>	0.328±0.184 ^a	1.06±0.017 ^a	0.126±0.063 ^b	.	2.03±0.012	0.713±0.023 ^b	0.997	0.000	<.0001
	<i>10LPS</i>	0.260±0.079 ^{ab}	0.928±0.024 ^a	0.227±0.001 ^a	.	2.03±0.008	0.793±0.039 ^a	0.972	0.001	<.0001
	<i>100LPS</i>	0.208±0.076 ^b	0.649±0.047 ^b	0.223±0.197 ^a	.	2.03±0.005	0.737±0.039 ^b	0.917	0.000	<.0001
Core Body Temperature (°C)	<i>Control</i>	0.889±0.469 ^c	0.327±0.048 ^a	0.147±0.025 ^b	.	3.33±0.010	39.9±1.05 ^b	40.44	0.028	<0.050
	<i>10LPS</i>	3.52±0.024 ^b	0.048±. ^b	0.226±0.050 ^a	0.752±0.023	3.33±0.012	40.0±1.19 ^a	41.06	0.198	0.1754
	<i>100LPS</i>	6.09±0.493 ^a	0.015±0.001 ^c	0.290±0.006 ^a	0.644±0.014	8.58±0.081	39.8±1.10 ^b	40.95	0.214	0.1249

A: Represents the amplitude of change in the response variable.

k₁: The rate constant for the initial increase phase.

k₂: The rate constant for the decay phase.

t₀: The time shift parameter for the initial increase phase.

t₁: The time shift parameter for the decay phase.

C: The asymptotic value or minimum response variable at $t=\infty$.

^{a, b, c} in the same column represents a significant difference among treatments for P value <0.05.

Parameters containing (.) that do not present statistical significance are considered $t_0=0$.

[†]Peak value was estimated based on the model for $t=t_0$.

Table 2. Results of serum extract pathway analysis from comparisons between **CON**, **10LPS** and **100LPS** treatments in broiler chickens

Pathway name	TC	Hits	Raw <i>p</i>	FDR
CON vs. 10LPS				
<i>Threonine and 2-Oxobutanoate Degradation</i>	20	1	0.029	0.509
<i>Arginine and Proline Metabolism</i>	52	9	0.03	0.509
<i>Oxidation of Branched Chain Fatty Acids</i>	26	1	0.036	0.509
<i>Mitochondrial Electron Transport Chain</i>	19	1	0.036	0.509
<i>Phytanic Acid Peroxisomal Oxidation</i>	26	1	0.036	0.509
<i>Propanoate Metabolism</i>	42	3	0.047	0.536
CON vs. 100LPS				
<i>Propanoate Metabolism</i>	42	3	0.005	0.302
<i>Cysteine Metabolism</i>	26	1	0.014	0.302
<i>Arachidonic Acid Metabolism</i>	67	1	0.014	0.302
<i>Valine, Leucine and Isoleucine Degradation</i>	69	6	0.017	0.302
<i>Lysine Degradation</i>	30	2	0.023	0.317
<i>Ketone Body Metabolism</i>	13	2	0.035	0.323
<i>Tryptophan Metabolism</i>	59	4	0.039	0.323
<i>Folate Metabolism</i>	29	2	0.046	0.323
<i>Glucose-Alanine Cycle</i>	13	3	0.052	0.323
10LPS vs. 100LPS				
<i>Alanine Metabolism</i>	17	3	0.089	0.998
<i>Histidine Metabolism</i>	42	2	0.091	0.998

TC: the total number of compounds in the pathway.

Hits: the actual matched number from the user uploaded data.

Raw *p*: the original p-value calculated from the enrichment analysis.

FDR: false discovery rate.

CON: control non-challenged birds.

10LPS: challenged birds with 10 µg LPS/kg BW.

100LPS: challenged birds with 100 µg LPS/kg BW.

Table 3. Results of liver extract pathway analysis from comparisons among **CON**, **10LPS**, and **100LPS** treatments in broiler chickens

Pathway name	TC	Hits	Raw p	FDR
CON vs. 10LPS				
<i>Bile Acid Biosynthesis</i>	65	3	<0.001	0.01
<i>Methionine Metabolism</i>	42	5	<0.001	0.013
<i>Porphyrim Metabolism</i>	40	1	0.006	0.151
<i>Phosphatidylethanolamine Biosynthesis</i>	12	2	0.008	0.158
<i>Phospholipid Biosynthesis</i>	29	2	0.017	0.215
<i>Phosphatidycholine Biosynthesis</i>	14	2	0.017	0.215
<i>Carnitine Synthesis</i>	22	4	0.021	0.221
<i>Betaine Metabolism</i>	21	3	0.025	0.228
<i>Glutathione Metabolism</i>	20	4	0.034	0.247
<i>Catecholamine Biosynthesis</i>	20	2	0.036	0.247
<i>Alanine Metabolism</i>	17	3	0.04	0.247
<i>Retinol Metabolism</i>	35	1	0.04	0.247
CON vs. 100LPS				
<i>Ketone Body Metabolism</i>	13	2	0.007	0.194
<i>Oxidation of Branched Chain Fatty Acids</i>	26	2	0.007	0.194
<i>Phytanic Acid Peroxisomal Oxidation</i>	26	2	0.007	0.194
<i>Butyrate Metabolism</i>	19	1	0.013	0.236
<i>Carnitine Synthesis</i>	22	4	0.016	0.236
<i>Betaine Metabolism</i>	21	3	0.019	0.236
<i>Bile Acid Biosynthesis</i>	65	3	0.036	0.353
<i>Mitochondrial Electron Transport Chain</i>	19	2	0.038	0.353
<i>Methionine Metabolism</i>	42	5	0.05	0.41
10LPS vs. 100LPS				
<i>Ketone Body Metabolism</i>	13	2	0.024	0.333
<i>Glycine and Serine Metabolism</i>	59	11	0.029	0.333
<i>Fatty Acid Biosynthesis</i>	35	3	0.03	0.333
<i>Alanine Metabolism</i>	17	4	0.045	0.333
<i>Methionine Metabolism</i>	42	5	0.047	0.333
<i>Ammonia Recycling</i>	31	7	0.052	0.333

TC: the total number of compounds in the pathway.

Hits: the actual matched number from the user uploaded data.

Raw p: the original p-value calculated from the enrichment analysis.

FDR: false discovery rate.

CON: control non-challenged birds.

10LPS: challenged birds with 10 µg LPS/kg BW.

100LPS: challenged birds with 100 µg LPS/kg BW.

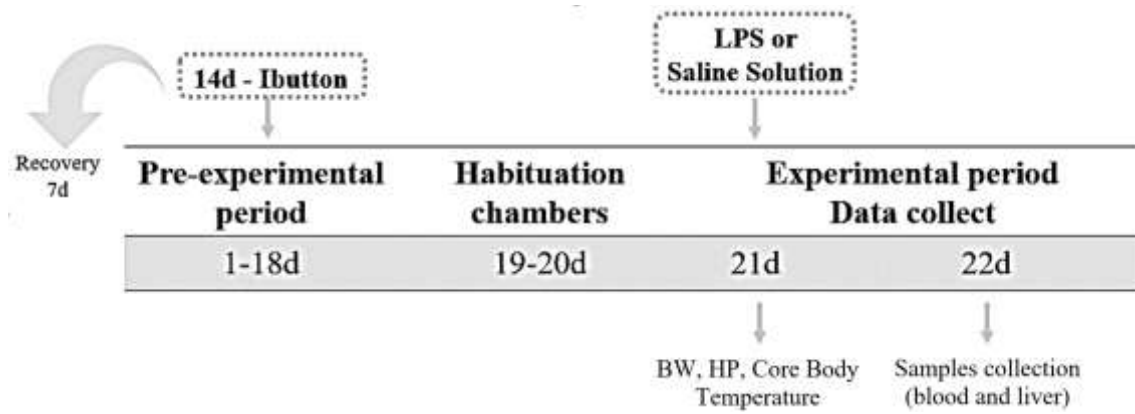


Figure 1. Protocol and data collection procedure. Seven days before (14d) the experiment, a datalogger for body temperature measurements was surgically implanted. After a phase of habituation inside the respirometric chamber (19-20d). On the 21d, chicks were injected with LPS (10 and 100 $\mu\text{g/kg}$) or saline and data were collected for 23h. HP: heat production.

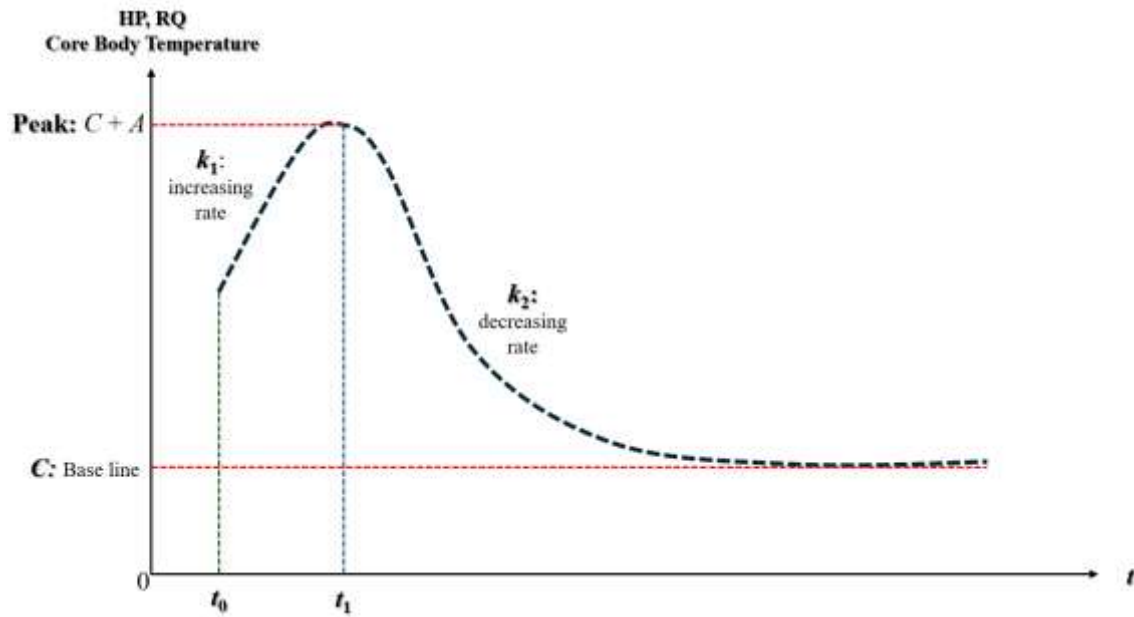


Figure 2. Illustration of the parameters determined for each response variable (HP- heat production, RQ- respiratory quotient or core body temperature) as a function of time. **A**: Represents the amplitude of change in the response variable. **k₁**: The rate constant for the initial increase phase. **k₂**: The rate constant for the decay phase. **t₀**: The time shift parameter for the initial increase phase. **t₁**: The time shift parameter for the decay phase. **C**: The asymptotic value or minimum response variable at $t=\infty$.

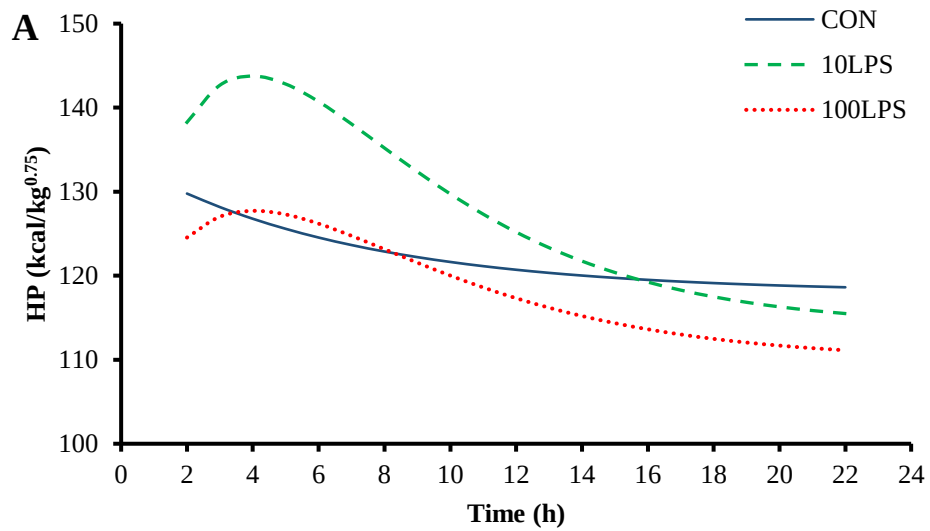
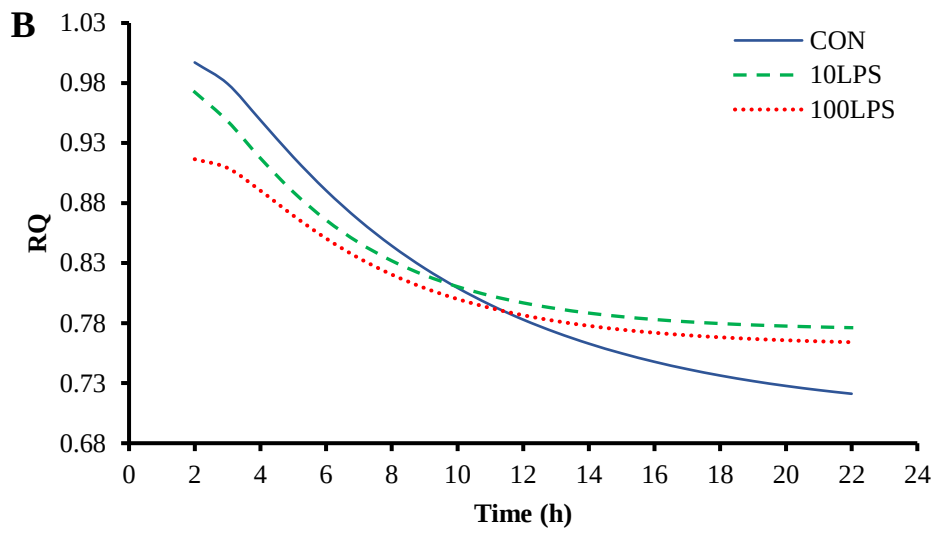
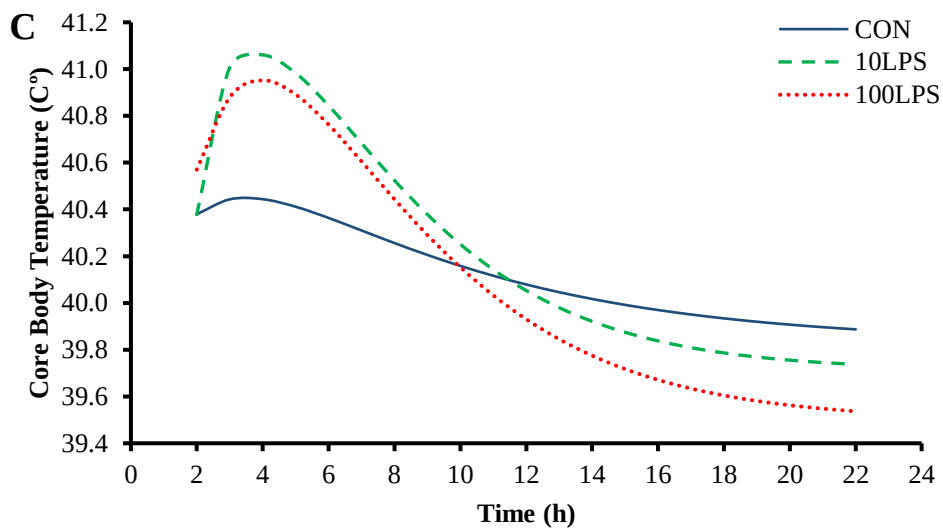
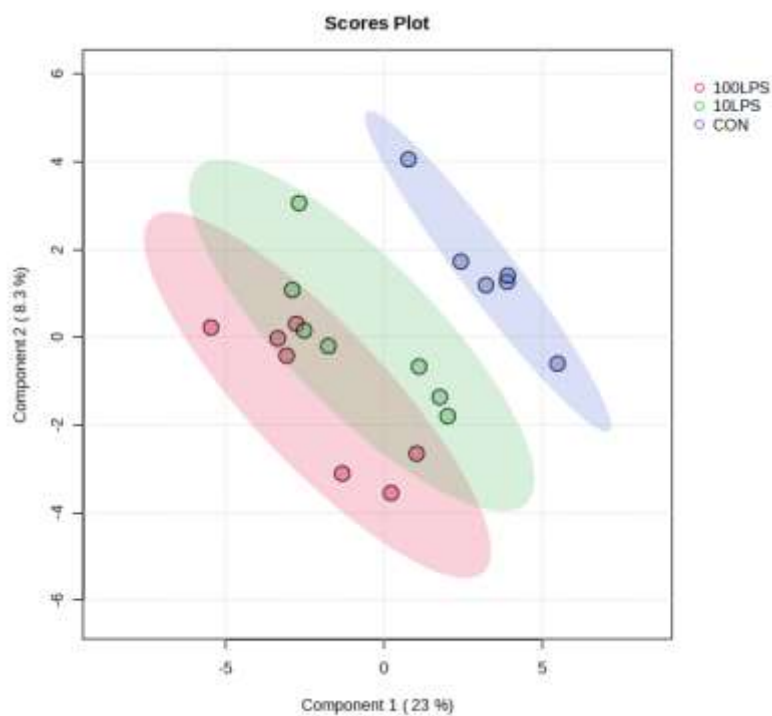
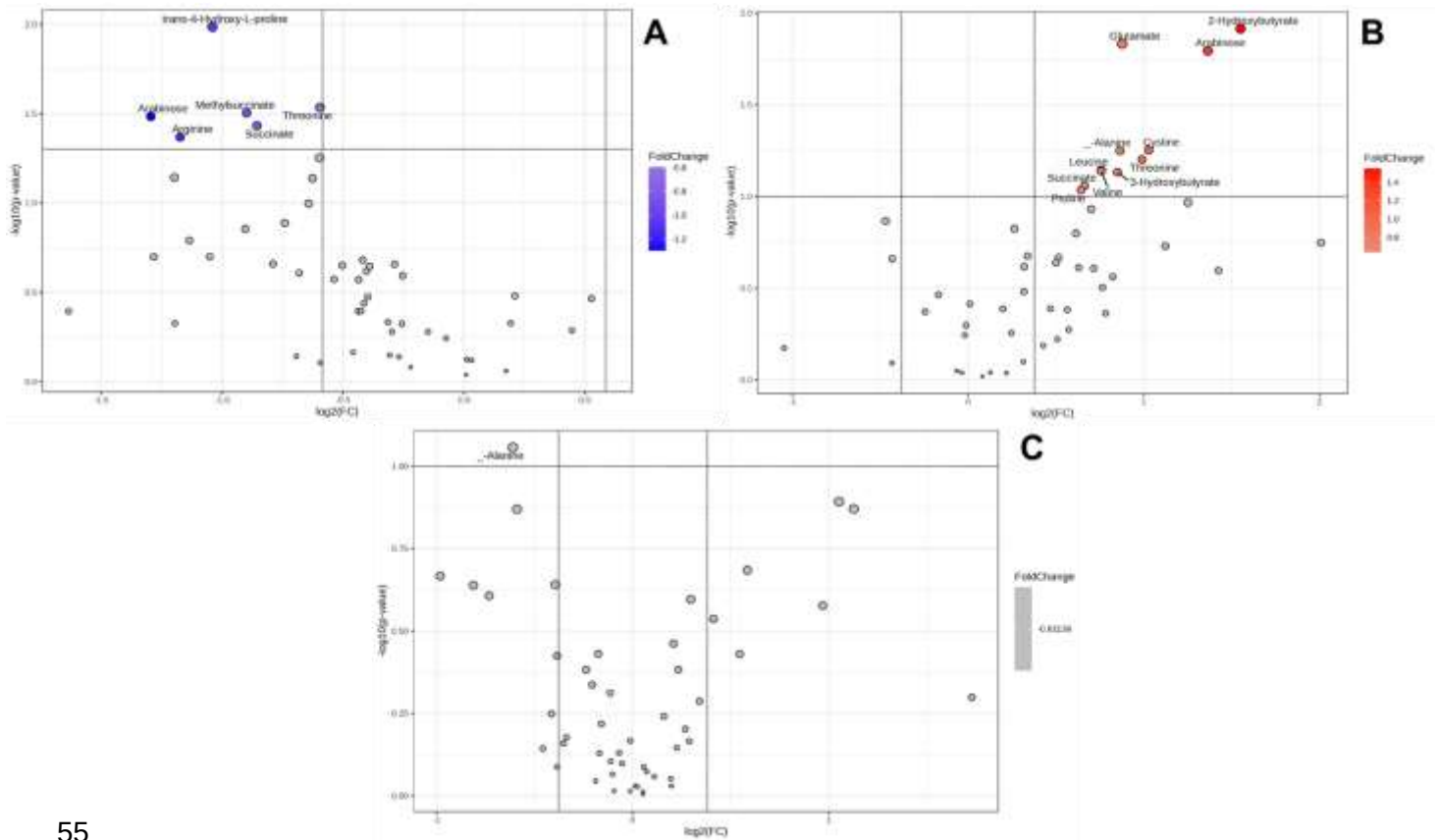


Figure 3. Graphical description of predicted values for the variables Heat Production (A), Respiratory Quotient (B), and Core Body Temperature (C).



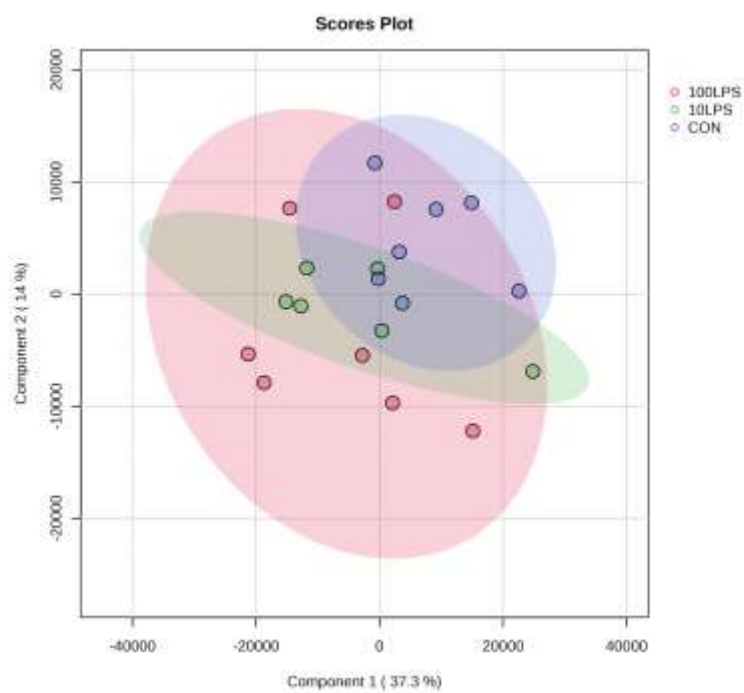
50

51 **Figure 4.** PLS-DA score plot of metabolite profiles in broiler serum. Abbreviations: PLS-
 52 DA, partial least squares discriminant analysis; **CON**, control non-challenged birds;
 53 **10LPS**, challenged birds with 10 µg LPS/kg BW; **100LPS**, challenged birds with 100 µg
 54 LPS/kg BW.



55

56 **Figure 5.** Volcano plots: (A) **CON** vs. **10LPS**; (B) **CON** vs. **100LPS**; (C) **10LPS** vs.57 **100LPS**. Abbreviations: **CON**, control non-challenged birds; **10LPS**, challenged birds58 with 10 μg LPS/kg BW; **100LPS**, challenged birds with 100 μg LPS/kg BW.



59

60 **Figure 6.** PLS-DA reception plot of metabolite profiles in broiler Liver. Abbreviations:
 61 PLS-DA, partial least squares discriminant analysis; **CON**, control non-challenged birds;
 62 **10LPS**, challenged birds with 10 μg LPS/kg BW; **100LPS**, challenged birds with 100 μg
 63 LPS/kg BW.

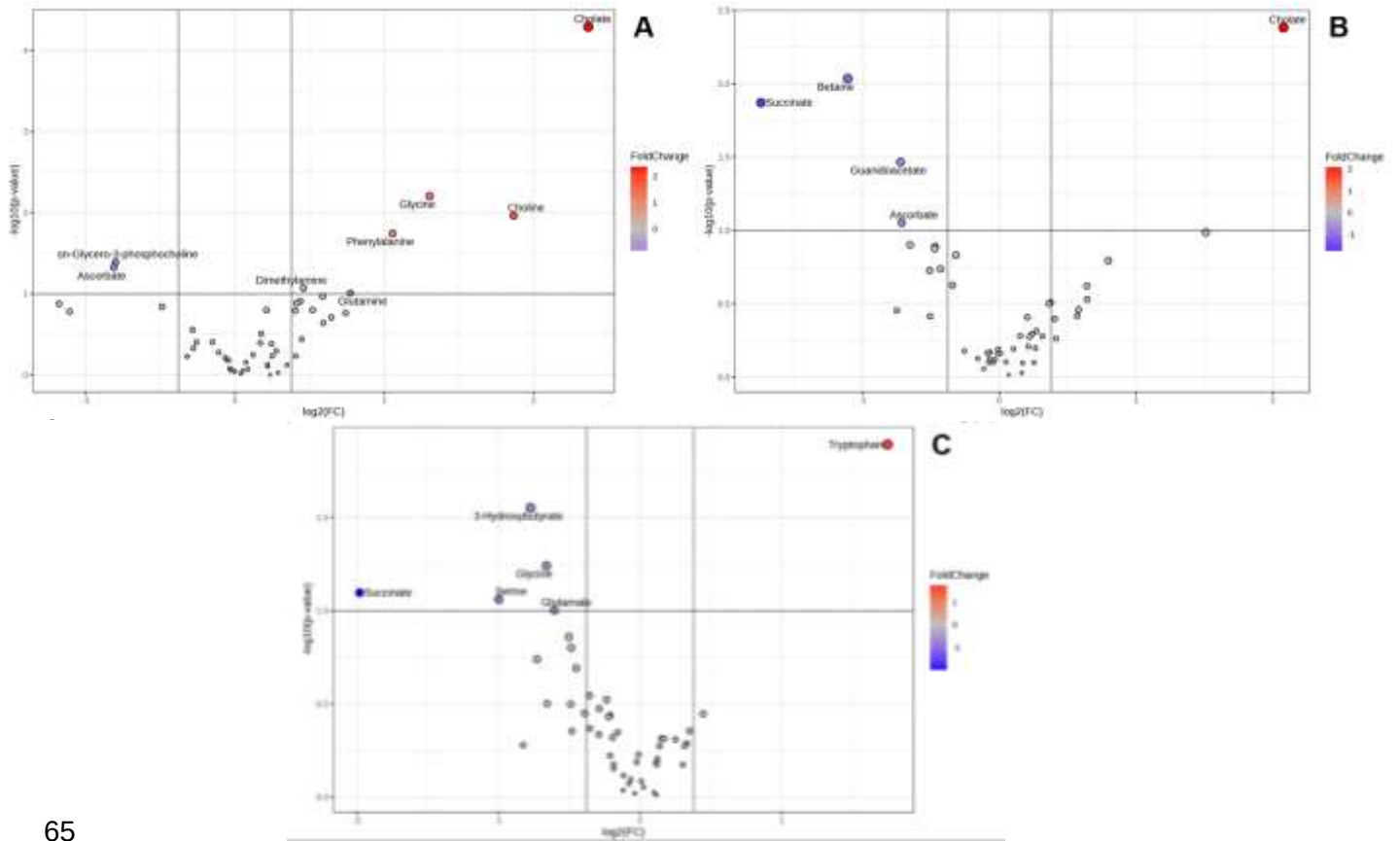
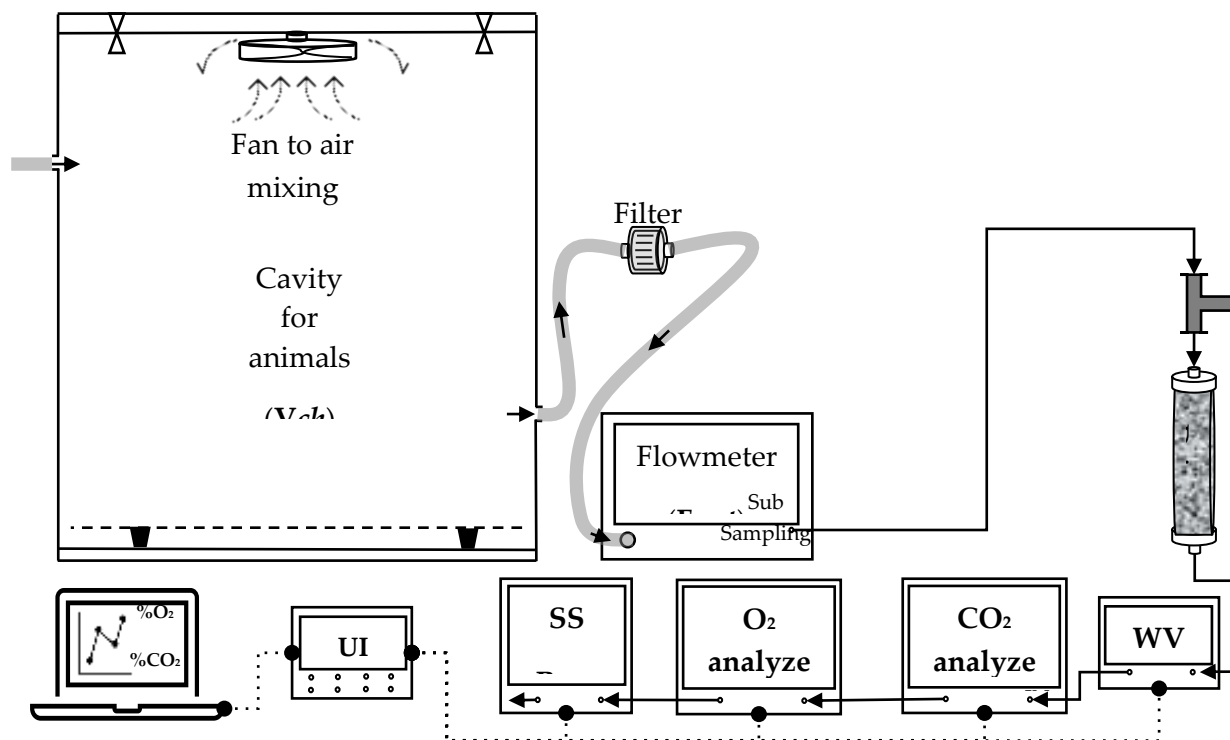


Figure 7. Volcano plots: (A) CON vs. 10LPS; (B) CON vs. 100LPS; (C) 10LPS vs. 100LPS. Abbreviations: CON, control non-challenged birds; 10LPS, challenged birds with 10 μg LPS/kg BW; 100LPS, challenged birds with 100 μg LPS/kg BW.

Supplementary material
Suppl. 1



Protocol for lack test of open-circuit chambers

This protocol is based on the principle of barometric pressure equilibrium by injection of a known volume of atmospheric gas.

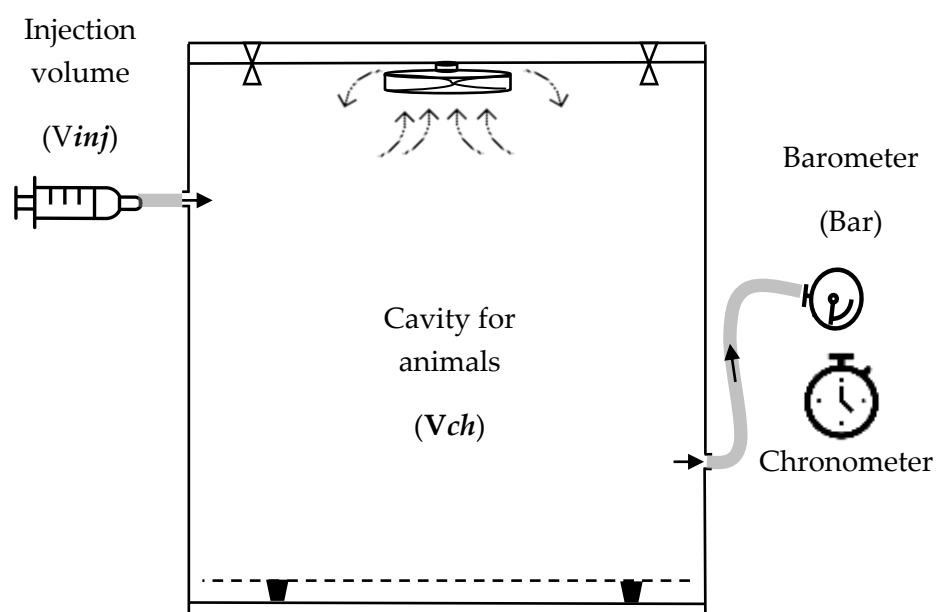


Figure 1. Scheme of lack-test of an open circuit chamber

90 **Table 1.** Variables and parameters of the lack-test.

Variables	Abbreviation	Value	Units
Internal volume of the chamber	Vch	100	L
Injected volume	Vinj	2.8	L
Pressure	Bar		kPa
Time	t	45	s

91

92 **Calculation**

93 - Injection volume rate (V_{inj}/V_{ch})

94 - Rate of lack (***F_{lack}***)

95
$$F_{lack} = \frac{V_{inj}}{V_{ch}} * \frac{1}{t \text{ (min)}}$$

96 **Result**

97 The rate of lack flow for the chamber 1, injection 2.8 L of atmospheric volume, with XX
98 barometric pressure, was:

99
$$F_{lack} = \frac{2.8}{100} * \frac{1}{\left(\frac{45}{60}\right)} = 0.0010 \text{ L/min}$$

CAPÍTULO 4 – Considerações Finais

Baseado nos achados descritos nos capítulos anteriores da presente tese, é possível verificar que as aves sob desafio sanitário apresentam alterações no seu metabolismo, e consequentemente uma maior demanda de energia para suportar o estresse imunológico causado pelo patógeno. A principal manifestação dessa condição de estresse é a redução no consumo de alimento voluntário, sendo o principal fator que leva a um menor ganho de peso. Este prejuízo na resposta produtiva é explicado pelo aumento na produção de calor para induzir a resposta febril, e principalmente pela redução da taxa de deposição de tecidos, e em casos de desafio crônico pelo catabolismo de reservas (gordura).

A partir dos pressupostos previamente descritos, é possível explorar que aves submetidas a um desafio agudo, cujo modelo de desafio foi por aplicação de LPS, apresentam um aumento de $19 \text{ kcal/kg}^{0.75}/\text{dia}$ de energia para cada variação de 1°C na temperatura corporal interna (Figura 1). Em contrapartida, aves submetidas a um desafio crônico, induzidas pelo modelo de desafio de enterite necrótica, apresentam um aumento de $58 \text{ kcal/kg}^{0.75}/\text{dia}$ de energia para cada variação de 1°C na temperatura corporal interna (Figura 1).

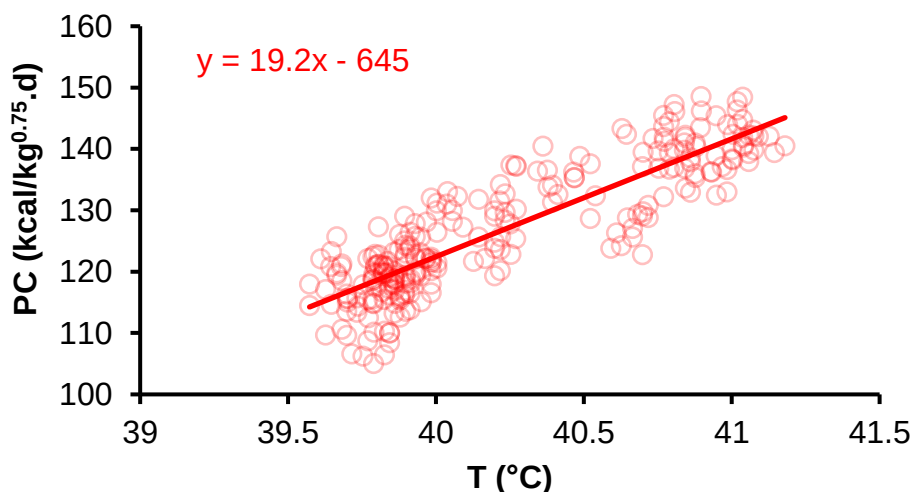


Figura 1. Relação da produção de calor e temperatura corporal interna de frangos de corte submetidos ao desafio com LPS.

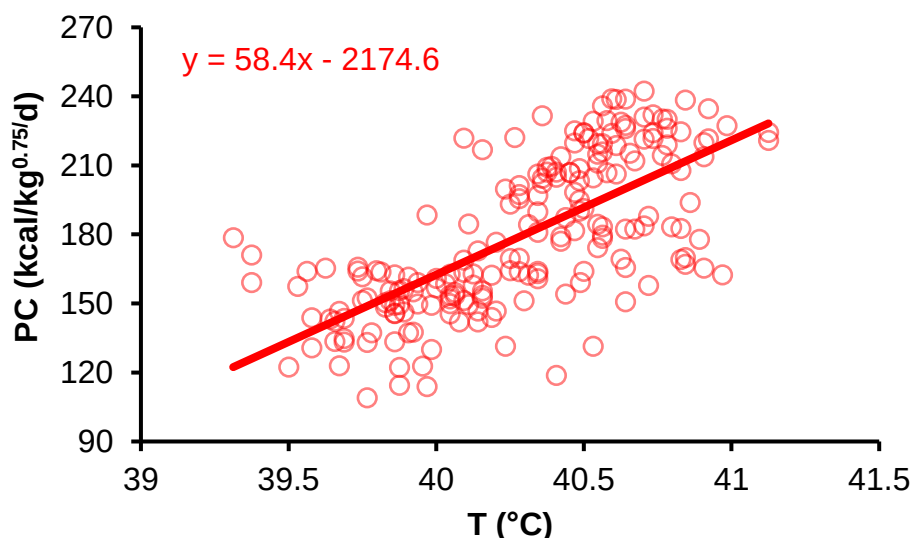


Figura 2. Relação da produção de calor e temperatura corporal interna de frangos de corte submetidos ao modelo de indução de Enterite Necrótica.

Evidenciando assim, que o desenvolvimento de febre é uma das respostas imunológicas não específicas de maior custo energético, sendo necessária uma exigência adicional, decorrente em condições de desafio crônico, do catabolismo endógeno, principalmente de reservas de gordura. Esse gasto extra de energia não pode ser totalmente compensado pelo aumento equiparado de energia na ração, pois as aves desafiadas apresentam um comportamento anoréxico, o que impossibilita o aumento do consumo alimentar. Este aumento da demanda energética das aves desafiadas, e a redução do consumo voluntário resulta num paradoxo entre a doença e a nutrição, o que impossibilita a aplicação de estratégias nutricionais para tentar amenizar os efeitos deletérios das condições do desafio. Nesse sentido, se faz necessário de um melhor entendimento da demanda energética das aves sobre estas condições.

Visualizando este cenário esboçamos um modelo fatorial, considerando as frações de manutenção, crescimento e a demanda energética para enfrentar o desafio sanitário. O modelo proposto foi o seguinte:

$$EMi = a * BW^{0,75} + c * BWG - n(\Delta \text{ taxa ganho})$$

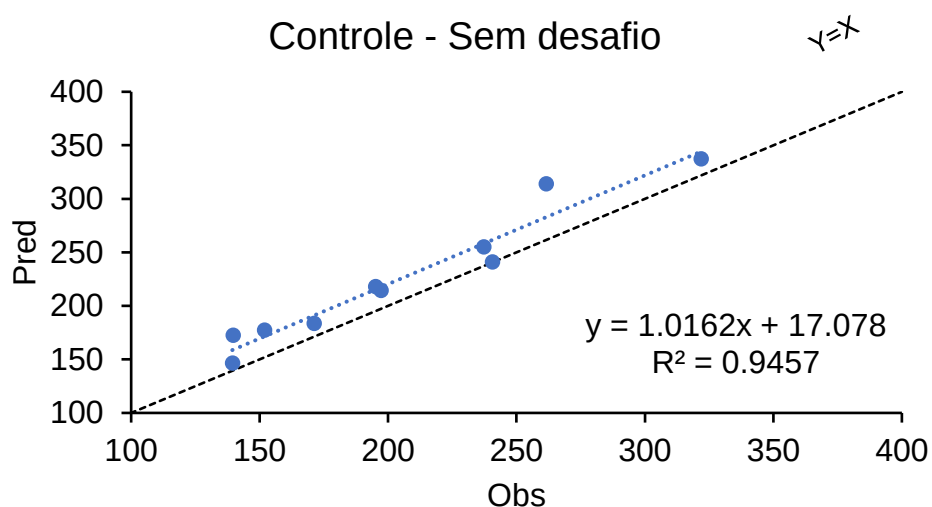
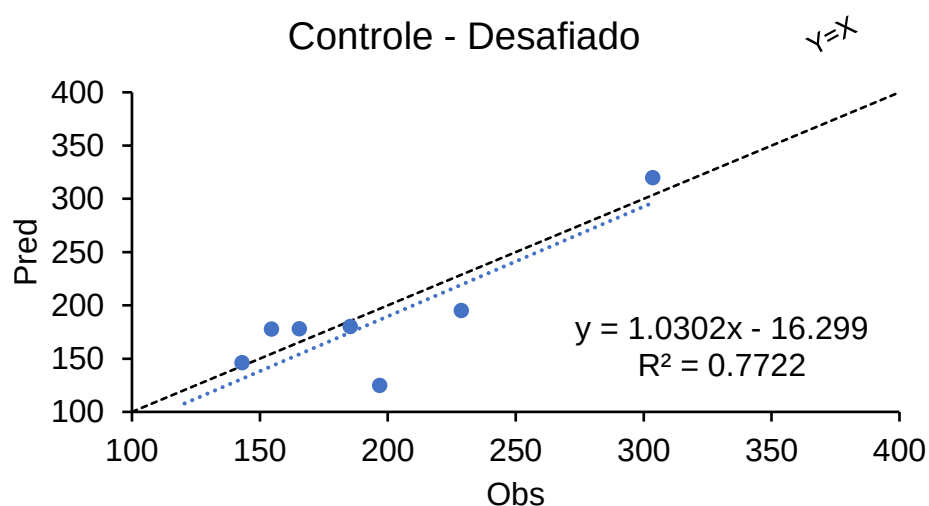
2,5
 kcal/g BWG
 ↑
 84
 kcal/ave
 ↓
 1,9
 kcal/g redução da taxa
 de crescimento
 ↓

Onde a representa a demanda energética para manutenção, c a exigência de energia para ganho de peso e n representa a demanda energética para o desafio sanitário, descrito como a diferença entre a taxa de ganho do grupo controle menos do grupo desafiado, representando “quanto a ave deixou de ganhar devido ao desafio”.

Este modelo foi desenvolvido com o intuito de estimar a demanda energética das aves sobre condições de desafio, e descrever uma condição hipotética quando são aplicadas as diferentes estratégias de uso de aditivos para amenizar os efeitos negativos em termos de utilização da energia.

Para a avaliação do modelo fatorial foi realizada a busca sistemática de trabalhos com frangos de corte sobre condições de desafio (Jia et al., 2009; Ritzi et al., 2014; Tian et al., 2016; de Oliveira et al. 2019; Granstad et al., 2020; Coles et al., 2021; Zhang et al., 2023; Barbalho et al., 2023; Bortoluzzi et al., 2024; Maria et al., 2024), incluindo diferentes estratégias do uso de aditivos.

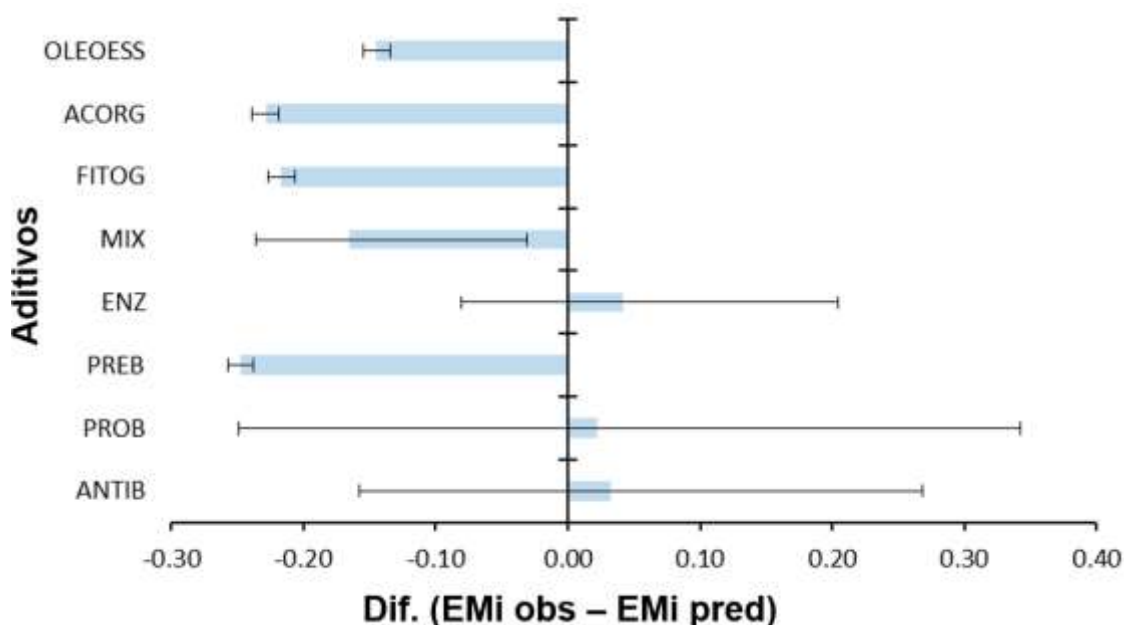
O primeiro passo na avaliação do modelo foi demonstrar o poder de
predição do modelo proposto nos grupos controle: não desafiado e desafiado:



Em abas avaliações, foi observado que os modelos apresentaram alto
poder de predição.

Baseado nisso foi avaliado o uso de aditivos que amenizem os prejuízos
causados pelo desafio e sejam substitutos aos antibióticos, como prebióticos,
probióticos, ácidos orgânicos, além de vitaminas e aminoácidos. O uso
estratégico de aditivos na alimentação de aves desafiadas sanitariamente pode
mitigar os efeitos negativos do estresse imunológico e das demandas

174 energéticas aumentadas. Ao melhorar a saúde intestinal, a digestão e a
 175 absorção de nutrientes, e ao modular a resposta imune, aditivos são
 176 fundamentais para manter a eficiência energética e promover o bem-estar e o
 177 desempenho das aves em condições de desafio.



178

179 O uso de antibióticos, probióticos e enzimas (carboidrases, glucanos, β -
 180 mamonase, etc.) apresentaram um maior valor de EMI observada em relação
 181 aos valores de EMI preditos, justificando seu uso para amenizar as reduções da
 182 EMI decorrentes ao desafio sanitário. Já os prebióticos, mix de aditivos,
 183 fitogênicos, ácidos orgânicos e óleos essenciais, apresentaram uma menor EMI
 184 observada em relação à EMI predita, o que sugere que estes aditivos nos
 185 trabalhos avaliados não foram suficientes para satisfazer as demandas
 186 energéticas das aves sobre estas condições.