

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

**EFEITO DA SUPLEMENTAÇÃO DE FITASE E DA FORMA
FÍSICA DA RAÇÃO NA UTILIZAÇÃO DA ENERGIA E
PRODUÇÃO DE CALOR EM FRANGOS DE CORTE**

Bruno Albino Leme
Zootecnista

2024

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PRODUÇÃO DE CALOR EM FRANGOS DE CORTE**

Bruno Balbino Leme

Orientadora: Profa. Dra. Nilva Kazue Sakomura

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

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IMPACTO POTENCIAL DESTA PESQUISA

Esta pesquisa visa esclarecer as respostas do metabolismo energético de frangos de corte recebendo dietas suplementadas com fitase e sobre a forma física da ração. Ao investigar como o aproveitamento energético das dietas frente a essas duas tecnologias empregadas, fitase e peletização, e suas respostas na retenção de energia no corpo, este estudo contribuirá para que os nutricionistas possam traçar estratégias com melhor entendimento da utilização da energia quando empregado essas tecnologias. Os insights obtidos nesta pesquisa podem colaborar com o avanço da nutrição de frangos de corte, promovendo uma maior rentabilidade e sustentabilidade da produção avícola.

POTENTIAL IMPACT OF THIS RESEARCH

This research aims to clarify the responses of energy metabolism of broilers receiving diets supplemented with phytase and the physical form of the feed. By investigating how the energy utilization of diets in relation to these two technologies employed, phytase and pelleting, and their responses in energy retention in the body, this study will help nutritionists to design strategies with a better understanding of energy utilization when using these technologies. The insights obtained in this research can contribute to the advancement of broiler nutrition, promoting greater profitability and sustainability of poultry production.

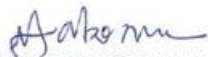
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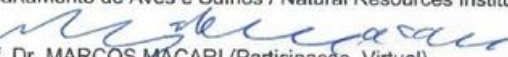
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DADOS CURRICULARES DO AUTOR

BRUNO BALBINO LEME, filho de Flávio Donizete Leme e Aparecida de Fátima Balbino Leme, nascido no dia 27 de novembro de 1995 em Mirassol, São Paulo. Ingressou no curso de Zootecnia na Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP) – Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, em fevereiro de 2013. Em fevereiro de 2018 obteve o título de Zootecnista. Em março de 2018 ingressou no Programa de Pós-Graduação em Zootecnia da Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP) – Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, sob orientação da Prof^a Dr^a Nilva Kazue Sakomura, obtendo o título de Mestre em Zootecnia em fevereiro de 2020. Em março de 2020 ingressou no Programa de Pós-Graduação em Ciência Animal da Universidade Estadual Paulista “Júlio de Mesquita Filho” (UNESP) – Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, sob orientação da Prof^a Dr^a Nilva Kazue Sakomura, submetendo a defesa da tese em junho de 2024.

Dedico

Aos meus pais Flávio e Aparecida, por todo amor, carinho e dedicação que sempre tiveram por mim, pelos esforços e incentivo durante toda minha trajetória. Amo cada um de vocês incondicionalmente.

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CEUA – COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Efeito da suplementação de enzimas e da forma física da ração na utilização da energia e na partição da produção de calor em frangos de corte"**, protocolo nº 5400/20, 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 11 de fevereiro de 2021.

Vigência do Projeto	03/07/2021 a 01/03/2023
Espécie / Linhagem	<i>Gallus gallus domesticus</i> / Cobb 500
Nº de animais	2976
Peso / Idade	40 gramas / 1 dia
Sexo	Macho
Origem	Incubatório Pluma, Descalvado – SP

Jaboticabal, 16 de fevereiro de 2021.


Profa. Dra. Fabiana Pilarski
Coordenadora – CEUA

EFEITO DA SUPLEMENTAÇÃO DE FITASE E DA FORMA FÍSICA DA RAÇÃO NA UTILIZAÇÃO DA ENERGIA E PRODUÇÃO DE CALOR EM FRANGOS DE CORTE

RESUMO – Objetivou-se com esta tese avaliar o efeito da suplementação de fitase e da forma física da ração na utilização de energia, partição da produção de calor, digestibilidade dos nutrientes, desempenho e rendimento de carcaça de frangos de corte. Os ensaios foram realizados no Laboratório de Ciências Avícolas da Faculdade de Ciências Agrárias e Veterinárias – UNESP, Campus de Jaboticabal, São Paulo. Para avaliar o efeito da suplementação de fitase foram realizados dois ensaios, um de desempenho, digestibilidade dos nutrientes, mineralização óssea, rendimento de carcaça e outro de metabolismo energético. No primeiro ensaio, foram avaliados dois controles negativos (CN) para 1000 e 1500FTU de fitase e a suplementação de fitase nos CN, com nove repetições de 30 aves cada, totalizando 1080 frangos de corte. Foram avaliados em quatro fases, 1-7 dias; 8-21 dias; 22-35 dias; e 36-42 dias, o desempenho das aves. Aos 21 dias foi determinado a digestibilidade do cálcio, fósforo, proteína e energia. Aos 21 e 42 dias foram avaliados o teor de cinzas, cálcio e fósforo da tíbia. Aos 23 e 41 dias foi mensurado a composição corporal, através do DEXA, e aos 43 dias, avaliado o rendimento de carcaça e cortes. Os CN apresentaram redução no consumo de ração, ganho de peso e piora na conversão alimentar, porém quando foi adicionado a fitase, em ambos os níveis houve melhora nesses parâmetros. A suplementação da fitase aumentou ($P<0,05$) a digestibilidade do fósforo, proteína e energia, e resultou em maior mineralização óssea das tíbias, assim como o conteúdo mineral ósseo mensurado pelo DEXA. Foi observado efeito ($P<0,05$) no rendimento de carcaça apenas para o grupo de 1500FTU de fitase. A suplementação de fitase melhorou o desempenho, digestibilidade e mineralização óssea de frangos de corte em dietas com redução de cálcio, fósforo e energia. No segundo ensaio, para avaliar o particionamento e utilização da energia, foram utilizados 160 frangos de corte de 11 a 20 dias. Os tratamentos consistiram em controle positivo, controle negativo (CN) com redução dos níveis nutricionais e o CN com 1000 e 2000 FTU de fitase. Foram mensurados a produção de calor total, consumo de ração e produção de excretas para o cálculo da energia metabolizável aparente (EMA) e corrigida pelo balanço de nitrogênio (EMAn). Para o cálculo da energia líquida (EL) da dieta, e a retenção de energia no corpo, foi utilizado um valor fixo do *Fasting heat production* de $107\text{kcal/kg}^{0,75}$. A energia retida (ER) no corpo foi determinado em ER como proteína e gordura. Foi observado efeito ($P<0,05$) na ingestão de EL, ER total e ERp e ERg, o CP apresentou valores superiores ao CN e quando se adicionou os dois níveis de fitase houve um maior conteúdo de EL da dieta, e maior retenção de energia no corpo, igual ao CP. O RQ (quociente respiratório) apresentou menor valor ($P<0,05$) para o CN em relação aos demais tratamentos. O sistema de EL foi capaz de detectar diferenças evidenciando a melhora do aproveitamento energético de dietas suplementadas com fitase em frangos de corte. Para avaliar o efeito da forma física da ração, foi realizado um ensaio com seis tratamentos com quatro repetições de oito aves cada, para o metabolismo energético, sendo ração: farelada, peletizada, finos, peletes + finos em comedouros separados e peletes + finos misturados, e um

tratamento pair feeding em pelete do tratamento farelado. Foram utilizados 192 frangos de corte de 17 a 26 dias. Os ensaios foram realizados em câmaras respirométricas para mensurar a produção de calor total, consumo de ração, produção de excretas e calculado os valores de EMA, EMAn, EL, RE e incremento calórico (IC). Foi mensurado o peso da moela, proventrículo, intestino, fígado e gordura abdominal de duas aves por parcela no final do ensaio. Para os tratamentos que receberam as dietas pelete + finos separados ou misturado foi realizado uma análise de preferência alimentar. Foi observado melhora na disponibilidade e utilização da energia das aves que receberam peletes, o que resultou em maior teor de ER, principalmente como gordura. Também apresentaram maior peso relativo de gordura abdominal, fígado, intestino e proventrículo. As aves tiveram maior preferência por pelete quando foi fornecido pelete e finos misturados do que em comedouros separados. Concomitantemente a esse ensaio, foi realizado um ensaio de desempenho em galpão com os mesmos tratamentos, exceto o *pair feeding* do ensaio de metabolismo. Foram utilizados 1500 frangos de corte machos de 11 a 42 dias, divididos em duas fases, 11 a 21 e 22 a 42 dias. Foram avaliados consumo de ração e peso corporal, e calculado ganho de peso e conversão alimentar. Aos 42 dias, foi avaliado rendimento de carcaça e cortes e realizado abate comparativo para determinar a deposição de proteína e gordura corporal. Foi observado maior ($P<0,05$) consumo de ração, ganho de peso e melhor conversão alimentar nas aves que receberam pelete. Neste ensaio, as aves que tiveram maior preferência por peletes foram as que receberam peletes e finos em comedouros separados. O rendimento de carcaça não foi afetado pelos tratamentos, e a deposição de proteína e gordura não apresentou efeito ($P<0,05$), porém foi observado maior conteúdo de gordura abdominal.

Palavras-chave: Energia líquida, energia retida, peletização, respirometria

EFFECT OF PHYTASE SUPPLEMENTATION AND PHYSICAL FORM OF THE FEED ON THE USE OF ENERGY AND HEAT PRODUCTION IN BROILER CHICKENS

ABSTRACT – The aims of this thesis were to evaluate the effects of phytase supplementation and the physical form of feed on energy utilization, heat production partitioning, nutrient digestibility, performance, and carcass yield of broiler chickens. The trials were conducted at the Poultry Sciences Laboratory of the Faculty of Agricultural and Veterinary Sciences – UNESP, Jaboticabal Campus, São Paulo. To evaluate the effect of phytase supplementation, two trials were carried out: one for performance, nutrient digestibility, bone mineralization, and carcass yield, and another for energy metabolism. In the first trial, two negative controls (NC) were evaluated for 1000 and 1500 FTU of phytase matrix of nutrients, with and without phytase supplementation, with nine replications of 30 birds each, totaling 1,080 broiler chickens. They were evaluated in four phases: 1-7 days, 8-21 days, 22-35 days, and 36-42 days, assessing bird performance. At 21 days, the digestibility of calcium, phosphorus, protein, and energy was determined. At 21 and 42 days, the ash, calcium, and phosphorus content of the tibia were evaluated. At 23 and 41 days, body composition was measured using DEXA, and at 43 days, carcass and cuts yield were evaluated. The NC showed reduced feed intake, body weight gain, and worsened feed conversion ratio. However, phytase supplementation at both levels improved these parameters. Phytase supplementation increased ($P<0.05$) the digestibility of phosphorus, protein, and energy, leading to greater bone mineralization of the tibias, as well as higher bone mineral content measured by DEXA. An effect ($P<0.05$) on carcass yield was observed only in the 1500 FTU phytase group. Phytase supplementation improved performance, digestibility, and bone mineralization in broiler chickens on diets with reduced calcium, phosphorus, and energy. In the second trial, 160 broiler chickens aged 11 to 20 days were used to evaluate energy partitioning and use. The treatments included positive control (PC), negative control (NC) with reduced nutritional levels, and NC with 1000 and 2000 FTU of phytase. Total heat production, feed intake, and excreta production were measured to calculate apparent metabolizable energy (AME) and AME corrected by nitrogen balance (AMEn). To calculate the net energy (NE) of the diet and energy retention in the body, a fixed fasting heat production value of $107 \text{ kcal/kg}^{0.75}$ was used. Retained energy (RE) in the body was determined in RE as protein and fat. An effect ($P<0.05$) was observed on the intake of NE, total RE, RE as protein (REp), and RE as fat (Ref). The PC presented higher values than the NC, and when phytase was added, there was a greater content of NE in the diet and greater retention of energy in the body, comparable to PC. The respiratory quotient (RQ) was lower ($P<0.05$) for NC compared to the other treatments. There were differences in the NE, showing improved energy utilization in diets supplemented with phytase in broiler chickens. To evaluate the effect of the physical form of the feed, a trial was conducted with six treatments, each with four replications of eight birds, for energy metabolism: mash, pellet, fines, pellets + fines in separate feeders, pellets + fines mixed, and a pair feeding treatment in pellets of the mash treatment. 192 broiler chickens aged 17 to 26 days were used. The trial was conducted in respirometric chambers to measure total heat production, feed intake, and excreta production, and to calculate the values of AME, AMEn, NE, RE, and heat increment

(HI). The weights of the gizzard, proventriculus, gut, liver, and abdominal fat of two birds per unit were measured at the end of the trial. For treatments that received separate or mixed pellet + fines diets, a food preference analysis was carried out. Improved energy availability and use were observed in birds that received pellets, resulting in a higher RE content, mainly as fat. They also presented greater relative weights of abdominal fat, liver, gut, and proventriculus. Birds showed a greater preference for pellets when pellets and fines were provided mixed rather than in separate feeders. Concomitantly with this trial, a performance trial was conducted in a barn with the same treatments, except for pair feeding in the metabolism trial. 1500 male broilers aged 11 to 42 days were used, divided into two phases: 11 to 21 and 22 to 42 days. Feed intake and body weight were evaluated, and body weight gain and feed conversion ratio were calculated. At 42 days, carcass and cuts yield were evaluated, and comparative slaughter was conducted to determine protein and fat deposition in the body. Greater ($P<0.05$) feed intake, weight gain, and better feed conversion ratio were observed in birds that received pellets. Birds showed a greater preference for pellets when pellets and fines were provided in separate feeders. Carcass yield was not affected by the treatments, and protein and fat deposition showed no significant effect ($P<0.05$), although a higher abdominal fat content was observed.

Keywords: Net energy, retained energy, pelletization, respirometry

CAPÍTULO 1 - Considerações gerais

1. Introdução

O Brasil ocupa a segunda posição mundial em produção de carne de frango (14,524 milhões de toneladas) e a primeira (4,822 milhões de toneladas) como exportador (ABPA, 2023). É certo a influência da genética, nutrição e ambiência na produtividade para a obtenção de melhores resultados no desempenho e na qualidade no produto final. Sabe-se que o custo da alimentação de frangos de corte corresponde à maior fração do custo final de produção, portanto o desenvolvimento de pesquisas na área de nutrição com objetivo de gerar programas com maior eficiência alimentar e de reduzir tais custos são de grande importância para a cadeia produtiva de frangos de corte.

Tendo em vista que o consumo de ração de aves é regulado principalmente para atender suas necessidades energéticas, uma estimativa acurada de energia em relação às exigências da ave e ao valor nos ingredientes usados na formulação de dietas, baseados em um mesmo sistema, é importante para traçar estratégias nutricionais mais precisas, de menor custo e favorecer a oferta de alimento adequada para atender as necessidades energéticas dos animais. Embora o sistema de energia metabolizável (EM) tenha sido comumente utilizado para formular dieta para aves, ele apresenta certas limitações, incluindo a não contabilização da perda de energia pela produção de calor.

O sistema de energia líquida (EL) tem sido sugerido como o mais acurado para expressar tanto a exigência energética das aves quanto o valor energético dos alimentos, pois considera as diferenças na eficiência de utilização da EM das dietas (Noblet, 2007). Essa característica é importante, pois permite incluir ingredientes de diferentes origens e composições nutricionais na formulação de rações sem que prejudique a precisão do valor energético das dietas. Estas vantagens permitem ajustar o fornecimento de energia para as aves reduzindo os efeitos do estresse térmico e custos com a alimentação. Mesmo considerando todos os benefícios de sua implementação, o sistema de EL não tem sido usado na formulação de dietas comerciais para aves, pois ainda não está bem estabelecido com uso prático como, por exemplo, é para suínos.

A energia ingerida decorrente da oxidação dos nutrientes da dieta é utilizada pelas aves para diferentes funções metabólicas e deposição de tecidos, porém existem variações na composição nutricional da dieta que afetam a digestibilidade e a eficiência de utilização da energia, portanto, uma fração dos nutrientes não é totalmente aproveitada. É bem conhecido que o uso de enzimas exógenas melhora a digestibilidade e aproveitamento dos nutrientes, principalmente as fitases, favorecendo uma maior liberação de cálcio, fósforo e energia, tendo como resultado melhoras na resposta produtiva das aves (Cowieson, 2005; Cowieson et al., 2019). Outro fator que pode alterar o consumo e desempenho desses animais é o processo de peletização da ração, que disponibiliza os nutrientes por desnaturação pelo cozimento parcial da dieta (Massuquetto et.al., 2019). Entretanto, existem lacunas em relação aos efeitos do uso de enzimas e o processo de peletização sobre a partição da EM. Sabe-se que o incremento calórico (IC) representa o custo energético da ingestão, digestão e atividade física, e é possível observar reduções no IC quando são aplicadas as estratégias mencionadas. Por outro lado, também pode ser evidenciada uma redução da energia líquida para manutenção (ELm) pela redução da demanda energética do metabolismo basal, em ambos os casos, esperando-se um aumento na deposição de tecido evidenciado no ganho de peso das aves.

2. Objetivos

- Avaliar os efeitos da suplementação de fitase exógena nos parâmetros de desempenho, mineralização óssea, digestibilidade dos nutrientes e da energia, utilização da energia e na partição da produção de calor em frangos de corte.
- Avaliar os efeitos do processo de peletização da ração nos parâmetros de desempenho, preferência alimentar, utilização da energia e na partição da produção de calor em frangos de corte.

3. Revisão de literatura

O conhecimento da energia e de sua importância para os processos fisiológicos levaram a insights fundamentais que culminaram no desenvolvimento de conceitos que foram a base para chegarmos nos sistemas de energia atuais utilizados para sua

predição nos alimentos e as necessidades energética dos animais. A utilização da energia da dieta tem sido avaliada por pesquisadores desde o século XV, entretanto, apenas em 1780, Lavoisier e Laplace relataram suas observações inovadoras em relação ao consumo de O₂, produção de CO₂ para o cálculo de oxidação dos componentes orgânicos e determinação do seu potencial calórico. Essas observações possibilitaram aos pesquisadores relacionarem o metabolismo e a combustão, através da relação estequiométrica da reação de oxidação de macronutrientes (ex. glicose):



Diante disso, a aceitação da teoria da combustão e metabolismo ainda levou tempo para dar continuidade nas investigações. Por volta do século XIX, novos objetivos foram propostos para se avaliar a energia utilizada na nutrição, a fim de estabelecer relações entre o consumo de O₂, produção de CO₂ e a liberação de calor com o que é consumido. Outro ponto foi gerar bases para avaliação dos alimentos, em relação ao conteúdo de energia, e como seriam os gastos para utilização desta energia.

As leis da termodinâmica, imprescindíveis na evolução dos sistemas de energia, foram postuladas em 1840, e a partir dessa data as pesquisas tiveram avanço significativo. A primeira lei, diz que a energia não pode ser criada e nem destruída, mas pode ser transformada, essa dedução foi crucial para sustentar a utilização da energia pelos animais como: EM = ER + PC, onde EM é a energia metabolizável que é fracionada em energia retida no corpo (ER), e produção de calor (PC) desprendida da atividade metabólica. A segunda lei, traz que todas as formas de energia podem ser convertidas quantitativamente em calor, sendo assim o calor químico perdido nas reações é independente do caminho seguido, ou seja, 1g de carboidrato oxidado em uma bomba calorimétrica originará a mesma quantidade de calor de 1g de carboidrato oxidado no organismo animal (Ferrell e Oltjen, 2008).

O conhecimento bioquímico de todas essas reações foi estudado e descrito na literatura, e foi muito importante para o entendimento do metabolismo energético animal (Lehninger et al., 2014). No tópico a seguir, iremos apresentar como essa

energia pode ser utilizada nas reações químicas que determinam o metabolismo que suportam as funções vitais de organismos complexos.

Além disso, renomados pesquisadores entre os que destacam Brody (1945), Kleiber (1961) e Blaxter (1962), trabalharam intensamente neste tema para melhor entender os fatores que pudessem influenciar nos processos metabólicos que envolvem a utilização de energia. Em 1965, Brouwer publica a equação para calcular a produção de calor, utilizado principalmente em avaliações nutricionais para animais de produção.

Os sistemas de predição de energia líquida para os animais foram então desenvolvidos, por diferentes métodos. A mensuração da produção de calor, por calorimetria direta ou indireta, possibilitou a mensuração da produção de calor no estado alimentado e em jejum, sendo possível separar o conteúdo de energia líquida de manutenção (ELm) e o incremento calórico (IC) (Noblet, 2007). Nesse sentido, utilizando a equação de Brouwer (1965), através do consumo de O₂ e produção de CO₂, pode ser calculado ambos os componentes. Por outro lado, outra forma que foi utilizada é a técnica do abate comparativo (Blaxter, 1966), que através da mensuração da EM e o conteúdo de energia que foi retida no corpo, tanto proteína quanto gordura, por diferença encontra-se a PC.

Diante disso, essa revisão tem como objetivo explorar os mecanismos de utilização da energia pelos animais, assim como reportar os sistemas de energia líquida desenvolvidos que culminaram no que se é utilizado atualmente. Além disso, trazer os desafios que ainda são encontrados para avaliação da energia dos alimentos e a interação com aditivos e tecnologias, como enzimas exógenas e o processamento de rações, e a utilização pelos animais.

3.1. Metabolismo energético

Os organismos celulares heterotróficos necessitam de energia transformada a partir do alimento consumido para se manterem em homeostasia, uma vez que não conseguem utilizar o carbono presente na atmosfera para gerar energia para o funcionamento das células e realização de trabalho, e utilizam carbono advindo de moléculas mais complexas como a glicose, sendo essa oriunda principalmente da quebra de carboidratos (Lehninger et al., 2014). Alguns carboidratos, como os

açúcares e amido, são os principais elementos da dieta de monogástricos, e a sua oxidação é a fonte primordial de energia (Józefiak et al., 2004).

Dentro de todo processo metabólico e em todas as transformações energéticas existe uma perda de energia livre e um aumento da energia não utilizável, desprendida do sistema como calor. Dessa forma, diferentemente do carbono, hidrogênio e nitrogênio, a energia não é reciclável, e segue o princípio da primeira lei da termodinâmica, que para qualquer mudança física ou química a quantidade total de energia se mantém constante, e que a energia pode mudar de forma ou ser transformada, porém não pode ser criada ou destruída.

O metabolismo, seja ele animal ou vegetal, pode ser definido de acordo com Lehninger et al. (2014), como o conjunto das transformações químicas que ocorrem na unidade celular ou no organismo por meio de uma série de reações catalisadas por enzimas que constituem as vias metabólicas. Dentro do metabolismo, temos a fase do catabolismo que é a degradação de nutrientes, na qual moléculas orgânicas complexas como carboidratos, lipídeos e proteínas são convertidas em produtos finais de moléculas simples ou monômeros, como ácido lático, CO_2 e NH_3 . As vias catabólicas liberam energia, e parte dessa energia é conservada na forma de ATP e de transportadores de elétrons reduzidos (NADH, NADPH e FADH_2), e o restante é perdido como calor, sendo então uma reação exergônica.

Por outro lado, a fase do anabolismo corresponde a biossíntese, onde precursores simples formam as moléculas maiores e complexas como lipídeos, ácidos nucleicos, polissacarídeos e proteínas. Entretanto, a fase anabólica necessita de energia para que as reações ocorram, e geralmente essa energia tem que estar na forma de potencial de transferência do grupo fosforil do ATP, e do poder redutor de NADH, NADPH e FADH_2 , sendo considerada uma reação endergônica (Lehninger et al., 2014).

Tanto o catabolismo quanto o anabolismo possuem vias metabólicas reguladas por enzimas, e que produzem alterações químicas que diferenciara os processos. Algumas dessas vias metabólicas são lineares e outras ramificadas, ou seja, geram diversos produtos finais úteis a partir de um único precursor ou convertem para uma vasta variedade de precursores em um único produto. De forma geral, no catabolismo as vias metabólicas são convergentes, em que precursores como glicogênio, amido,

triacilgliceróis, aminoácidos, entre outros, são convertidos em acetato (Acetil-CoA), e no anabolismo as vias metabólicas são divergentes, onde o Acetil-CoA é convertido em diferentes produtos como hormônios, triacilgliceróis, fosfolipídios, entre outros (Van Milgen, 2002). Ainda, algumas vias metabólicas podem ser cíclicas, onde um composto inicial da via é regenerado em uma série de reações que converte outro componente inicial em um produto, por exemplo o ciclo do ácido cítrico (Lehninger et al., 2014).

O mecanismo basal celular e suas funções vitais no organismo podem ser definidos como a taxa metabólica basal ou gasto energético para manutenção (Kleiber, 1961), onde o animal exige uma quantidade de energia e ainda parte dessa energia será perdida como calor em todo processo. Se o animal estiver em condições de restrição alimentar e o conteúdo de energia ingerido estiver abaixo das necessidades de manutenção, as vias catalíticas serão acionadas através da falta de substrato (aminoácidos ou ATP) para geração de energia. De forma contrária, se o conteúdo de energia for maior que as necessidades de manutenção as vias anabólicas serão acionadas e parte dessa energia será direcionada para geração de produtos finais que sustentarão a deposição dos tecidos.

3.2. Utilização do ATP como moeda energética

A adenosina trifosfato (ATP) é a principal molécula transportadora de energia dos seres vivos, e funciona também como depósito de energia, que quando necessário é acionada para utilização através de reações químicas de fosforilação. É constituída por um açúcar (ribose) ligado a uma base nitrogenada (adenina) e três grupos fosfatos em série.

As células heterotróficas obtêm energia livre de forma química pela quebra de moléculas de nutrientes, e elas usam essa energia para fazer ATP a partir de ADP e fosfato inorgânico (Pi). Então, a molécula de ATP doa parte da sua energia química para processos endergônicos, como por exemplo a síntese de metabólitos intermediários e de macromoléculas a partir de precursores menos complexos, para o transporte de substâncias através de membranas contra gradiente de concentração, e para o movimento mecânico. O processo de doar energia do ATP envolve sua

participação covalente na reação, com eventual conversão de ATP em ADP e Pi, ou ainda, em algumas reações, em AMP e 2 Pi (Lehninger et al., 2014).

A molécula de ATP, em organismos eucariotos, é produzida principalmente nas mitocôndrias, organelas responsáveis pela produção de energia e pelo processo de respiração celular. Neste processo, na presença de oxigênio (O_2) há a decomposição da matéria orgânica em dióxido de carbono (CO_2) e água (H_2O), sendo assim essa reação consome O_2 e produz CO_2 , sendo possível que a partir destes estimar a produção de calor dos organismos (Brouwer, 1965). Para cada molécula de glicose oxidada no processo de respiração celular é produzido cerca de 32 moléculas de ATP (Pfeiffer et al., 2001).

A liberação de energia oriunda da molécula de ATP pode ocorrer através de uma reação simples de hidrólise, onde na presença da molécula de H_2O é liberado um íon fosfato inorgânico (Pi) e energia através de uma reação exergônica, resultando em uma molécula de ADP ou AMP que poderá ser fosforilada e voltar a molécula de ATP. Entretanto, a maior parte de doação de energia por ATP envolve a transferência de grupos fosforil, e não simplesmente a hidrólise de ATP.

Sendo assim esse processo ocorre em duas etapas: (1) parte da molécula de ATP, ou seja, um grupo fosforil ou a porção adenilato (AMP) é primeiro transferida para uma molécula de substrato ou para um resíduo de aminoácido de uma enzima, tornando-se covalentemente acoplada ao substrato ou a enzima, aumentando dessa forma o conteúdo de energia livre; (2) a porção com fosfato transferida da primeira etapa é deslocada, gerando Pi, PPi ou AMP, então a molécula de ATP participa covalentemente da reação enzimática, gerando energia livre. Além disso, a transferência dos grupos fosforil do ATP ocorre somente quando estão presentes as enzimas específicas para reduzir a energia de ativação.

3.3. Cálculo da produção de calor através do $\dot{V}O_2$ e $\dot{V}CO_2$

A estequiometria é um ramo da química que calcula as quantidades de reagentes e/ou produtos de uma reação química. Esses cálculos são realizados com base nas leis das reações e na teoria atômica molecular, sendo elas: Lei das Proporções Constantes de Proust, que diz que quando várias substâncias se combinam para formar um composto, sempre o fazem em uma relação de massa definida; e a Lei da

Conservação da Massa de Lavoisier, que mostra que a massa total dos produtos em uma reação é sempre igual a massa total dos reagentes em um sistema fechado.

Existem variações em relação aos cálculos para estimar a produção de calor, entretanto, para estudos que utilizam a calorimetria indireta, têm-se utilizado a equação publicada por Brouwer (1965), que é baseada na mensuração do $\dot{V}O_2$, $\dot{V}CO_2$ e da produção de calor (Q), liberados na combustão de 1g de gordura, carboidrato e proteína. A partir desses dados, Brouwer (1965) concluiu que o $\dot{V}O_2$, $\dot{V}CO_2$ e o Q podem ser expressos em função das quantidades de proteína, gordura e carboidratos oxidados (equações 1, 2, 3 e 4).

- $Q(kj) = 16,175 \dot{V}O_2 (l) + 5,021 \dot{V}CO_2 (l) - 0,958 P(g)$ (1)

- Oxidação de carboidrato = $-2,965 \dot{V}O_2(l) + 4,170 \dot{V}CO_2(l) - 0,390 P (g)$ (2)

- Oxidação da gordura = $1,718 \dot{V}O_2(l) - 1,718 \dot{V}CO_2(l) - 0,315 P(g)$ (3)

- $Q(kj) = 16,175 \dot{V}O_2(l) + 5,021 \dot{V}CO_2 (l) - 0,958 P(g) - 2,167 VCH_4$ (4)

Onde, Q é a produção de calor em kJ, $\dot{V}O_2$ é o consumo de oxigênio em litros, $\dot{V}CO_2$ é a produção de dióxido de carbono, e P é a quantidade de proteína (nitrogênio multiplicado por 6,25) oxidada em gramas. A equação 1 não leva em consideração o gás metano (CH_4), e as equações 2 e 3 estimam a oxidação de carboidratos e gorduras, e a equação 4 possui a correção de CH_4 , que é mais utilizada para suínos e ruminantes por serem frações representativas na produção de gases decorrentes da respiração e fermentação.

Gerrits et al. (2015) relatam que a composição em proteína, carboidrato e gordura podem influenciar os coeficientes das equações, isso porque cada um destes substratos possui quantidades diferentes para oxidação de consumo de oxigênio, produção de dióxido de carbono, e liberação de calor. De acordo com Brouwer (1965) para oxidar 1g de carboidrato, por exemplo o amido, é consumido 0,829 (l) de O_2 , e produzido 0,829 (l) de CO_2 , gerando 17,57 KJ de calor. Para proteína, o consumo de O_2 é 0,957 (l), a produção de CO_2 0,774 (l) e gera 18,41 KJ de calor. Por fim, para gorduras o consumo de O_2 é de 2,013(l), produção de CO_2 de 1,431(l) e gera 39,75 KJ de calor. Além disso, outro parâmetro é utilizado para expressar a relação entre o consumo de O_2 e a produção de CO_2 que é o quociente respiratório (RQ), para carboidratos, proteínas e gorduras a relação é 1,00; 0,81; e 0,71 respectivamente.

Entretanto, dentro de cada substrato existem variações no consumo de O₂ e na produção de CO₂, Gerrits et al. (2015) traz um levantamento de dados, adaptado de Elia e Livesey (1988), e encontra variações do RQ de 0,81-0,87 para aminoácidos, e de 0,71-0,74 para gorduras. Além disso, o RQ pode trazer a informação de que substrato está sendo degradado, caso o animal esteja em jejum este quociente estará por volta de 0,71 a 0,87, indicando o catabolismo de gordura ou proteína corporal.

3.4. Sistema de energia líquida

A energia líquida (EL) é o sistema mais acurado para expressar o conteúdo energético de um alimento ou as necessidades energéticas dos animais para uma função fisiológica específica, entretanto, neste sistema também há dificuldades e desafios em se contabilizar todos os efeitos e sua magnitude (Kromman, 1973; Noblet, 2007), assim como para adotá-lo na formulação prática de alimentos.

As condições de avaliação da EL em diferentes sistemas podem levar a variações, mesmo que sejam pequenas, na resposta observada. Para contornar este problema, o sistema californiano (Lofgreen e Garrett, 1968), alemão (Nehring, 1969) e britânico (Blaxter, 1962), que são os sistemas que deram origem ao que é utilizado atualmente, assumiram que vários fatores eram constantes entre as condições em que os sistemas fossem utilizados, como por exemplo o ambiente.

Uma vez que a EL é a energia remanescente após descontar todas as perdas metabólicas, sendo: energia perdida nas fezes (energia digestível); energia perdida na urina e dos gases decorrente do processo digestível (energia metabolizável); e energia perdida pela produção de calor (incremento calórico), é a energia da ração que realmente é utilizada para manutenção e produção (figura 1), portanto mais sujeita a variações em sua determinação do que a energia digestível e metabolizável (Kromman, 1973). Dentre as perdas de energia, a maior porcentagem de perda, cerca de 20 a 60%, está atrelada a digestibilidade dos ingredientes, que é relacionado a composição da dieta, ou seja, esse montante de energia é perdido nas fezes. O esquema apresentado na figura 1 mostra a partição da energia e as suas perdas (Sakomura e Rostagno, 2016).

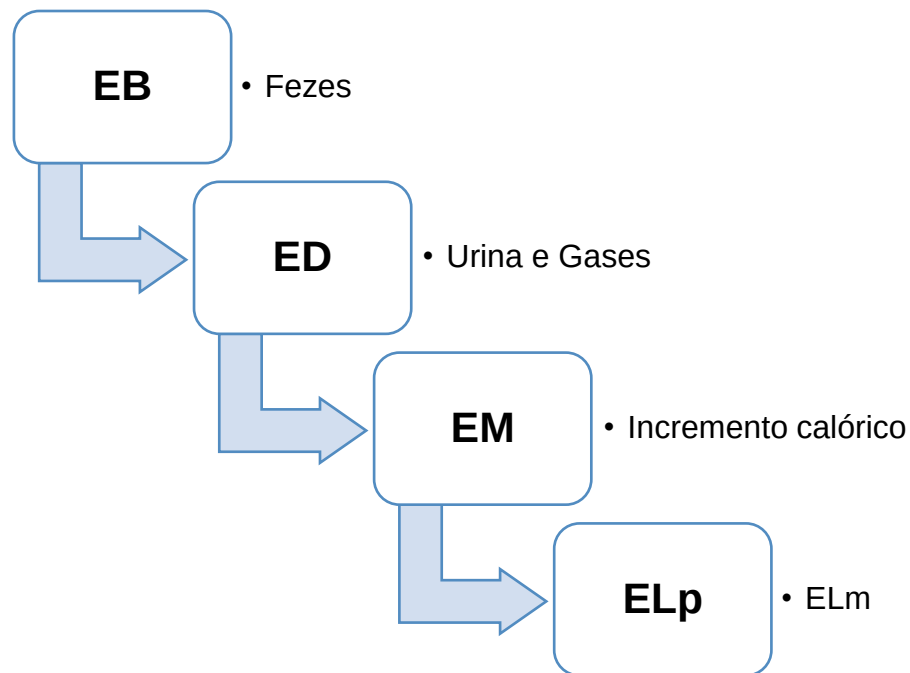


Figura 1. Representação da partição da energia

Farrel em 1974, descreveu detalhadamente a partição da energia para aves. Pelo fato de as aves excretarem as fezes e urina juntos é mensurado a energia metabolizável desprezando a energia digestível, ainda a produção de gases pelo trato gastrointestinal foi considerado desprezível para aves (Le Goff et al., 2002). A energia líquida é a EM corrigida pela produção de calor, que nesta época também era mensurado através do abate comparativo (Blaxter, 1966) para se obter a energia retida (ER), e utilizando a equação: $EM = ER + PC$, era possível encontrar a produção de calor.

No mesmo ano, De Groote (1974) publica um trabalhado com objetivo de comparar os sistemas de EM e EL na formulação com menor custo, e avaliaram a influência da composição da ração na eficiência de utilização da energia. Este autor concluiu que há diferença entre os sistemas EM e EL, e que o sistema de EL se mostrou mais eficiente para calcular dietas para frangos de corte, esse achado foi atribuído por levar em consideração a diferença nas eficiências de utilização da EM de cada ingrediente.

Além disso, De Groote (1974) elucida a diferença na eficiência de utilização das macromoléculas, carboidratos, proteínas e gorduras. O sistema de EM acaba

superestimando o conteúdo energético de ingredientes proteicos, e subestimando o conteúdo energético dos lipídeos. Isso porque a eficiência de utilização da EM para EL é menor para proteína do que para os lipídeos. Recentemente, Carré et. al. (2014) determinaram eficiências de utilização de frangos em crescimento de 79,8; 86,2 e 76% para carboidratos, gorduras e proteínas, respectivamente.

A aplicação do sistema de energia líquida para aves foi relativamente insignificante (Emmans, 1994) quando comparamos com outras espécies, sendo mais utilizado para ruminantes e de forma mediana para suínos (Noblet et al., 1994). Entretanto, alguns trabalhos (Cerrate et al., 2019; Cowieson et al., 2019; Wu et al., 2018; Teofilo et al., 2022; Riveros et al., 2022) demonstram que o setor avícola tem mostrado interesse em aplicar o sistema de EL nos últimos 10 anos, e Noblet et al. (2021) aponta uma perspectiva em aumentar as investigações nos próximos 15 anos.

3.5. Utilização de fitase em dietas para frangos de corte e o impacto na disponibilidade de energia

Normalmente, dietas para frangos de corte tem como base ingredientes de origem vegetal, e no Brasil têm sido utilizados principalmente o milho e farelo de soja. A maior parte do fósforo contido nesses ingredientes (cerca de 60-90% do fósforo total) está na forma de fitato que não é disponível para as aves (Sweeten, 1992). Dessa forma, fitases exógenas passaram a ser utilizadas na alimentação de aves comerciais com intuito de melhorar a eficiência de utilização do fósforo, outros minerais associados em forma de quelatos, aminoácidos, assim como da energia contida nos ingredientes (figura 2).

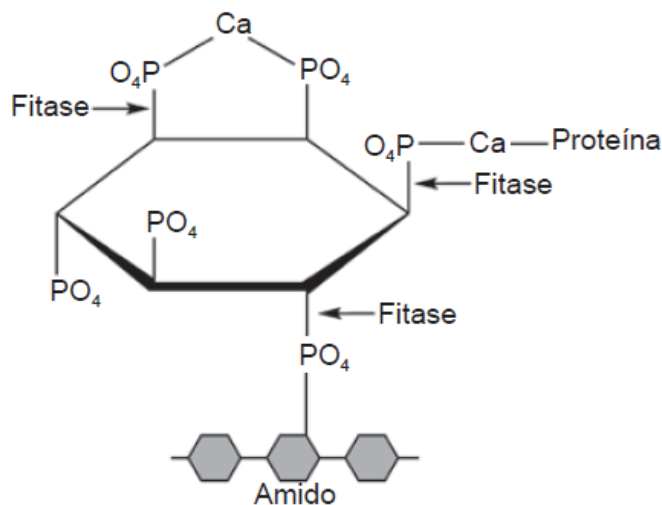


Figura 1. Esquema ilustrativo da atuação da fitase (Fonte: Romano e Russo, 2008)

A utilização de fitases exógenas tem sido avaliada na alimentação de frangos de corte proporcionando melhoria na digestibilidade e desempenho (Cowieson e Bedford, 2009; Walk et al., 2013; Cozannet et al., 2017; Cowieson et al., 2017; Cowieson et al., 2019). Walk e Poernama (2019) ao avaliarem dietas a base de milho e farelo de soja suplementadas com fitase (1500 FTU/kg ração) observaram efeito significativo no consumo de ração, ganho de peso e conversão alimentar, evidenciando a ação positiva da utilização de fitases. Dessimoni et al., (2020) ao também avaliarem dietas a base de milho e farelo de soja encontraram efeito significativo da suplementação de fitase no consumo de ração e ganho de peso de frangos de corte.

Além dos benefícios observados nos parâmetros de desempenho das aves quando é suplementado fitase em dietas de frangos de corte, outro ponto importante é em relação a mineralização óssea desses animais. Rutherford et al. (2012) ao avaliarem a suplementação de fitase em 1000 e 2000 FTU/kg, observaram uma melhora de 35% na mineralização da tíbia de frangos de corte, evidenciando o aumento da digestibilidade do fósforo e aumentando a deposição deste elemento no tecido ósseo. De acordo com Sakomura e Rostagno (2016) o conteúdo de cinzas na tíbia de frangos de corte é um ótimo indicativo de melhoria na digestibilidade e disponibilidade de fósforo. Além disso, a integridade óssea tem papel crucial na qualidade de carcaça, pois segundo Rath et al. (2000) no processamento da carcaça

se não houver qualidade no tecido ósseo, pode-se aumentar as perdas neste processo.

São vários os benefícios da fitase que estão descritos na literatura, no entanto, segundo Cowieson et al. (2017), os efeitos da fitase na energia não são relatados de forma consistente. Como mencionado nos tópicos anteriores, as necessidades energéticas são divididas em manutenção (ELm) e produção (ELp), sendo que a ELp pode ser depositada como proteína e gordura (Lopez e Leeson, 2008). A suplementação de fitase melhora a digestibilidade dos nutrientes, atuando na quebra do ácido fítico presente em ingredientes de origem vegetal capazes de hidrolisar um ou mais grupos de fosfato da molécula de inositol fosfato, hidrolisando fitatos que não se ligam apenas a P, Ca e outros micro minerais, mas também carboidratos e proteínas, liberando mais energia (Schöner e Hoppe, 2002; Gautier et al., 2018).

Entretanto, a digestibilidade influenciará principalmente no conteúdo da energia digestível, que para aves não é muito avaliada pela excreção da urina e fezes serem juntas, sendo assim o impacto será na EM. Porém, para se chegar no conteúdo de EL deve-se descontar a produção de calor, que por sua vez é dividido em manutenção (ELm) que é mensurada pela produção de calor em jejum, e o incremento calórico ou efeito térmico do alimento, calculada a partir da diferença entre PC e PCJ (Van Milgen et. al., 2001).

Dessa forma, um dos desafios para se avaliar a atuação da fitase exógena é elucidar em qual fração do calor produzido decorrente do uso metabólico do alimento a ação das enzimas se torna mais importante. Cowieson et al. (2019) ao avaliarem a EL de dietas contendo enzimas exógenas (Fitase, carboidrase e protease), mensuraram a produção de calor total, por calorimetria indireta utilizando câmaras respirométricas de circuito fechado, e considerou um valor fixo de ELm (450kj/kgPV^{0,70}) determinado por Noblet et al. (2015). Os autores observaram efeito significativo de forma positiva para o conteúdo de EL da dieta das aves que foram suplementadas com as enzimas exógenas.

3.6. Utilização de rações peletizadas para frangos de corte e o impacto na utilização da energia

A produção de frangos de corte é responsável por uma grande parcela da produção de rações em todo mundo. A alimentação destes animais corresponde a maior fração do custo de produção, entorno de 60 – 70%, e este custo está maioritariamente relacionado aos ingredientes, ainda assim, quando se utiliza de processamentos desta ração pode-se onerar mais o custo final da ração (Nolan et al., 2010). Entretanto, tais processos podem gerar benefícios a produção que garantem todo o investimento.

O processo de peletização é uma das tecnologias empregadas na produção de rações, cujo objetivo é transformar uma dieta farelada em peletes através da adição de vapor e a uma determinada pressão, gelatinizando parcialmente o amido, quebrando fatores antinutricionais, e formando um pelete integro e homogêneo que corresponde a uma dieta balanceada (Dozier et al., 2010). A desnaturação dos fatores antinutricionais possibilita a ação de enzimas endógenas no processo de digestão dos nutrientes, aumentando a digestibilidade dos mesmos (Jiménez-moreno et. al., 2016).

Diversos autores relatam os benefícios do processo de peletização de rações para frangos de corte, principalmente no que tange o consumo de ração (Abdollahi et. al., 2011; Chewning et. al., 2012; Abdollahi et. al., 2013; Mingbin et. al., 2015; Roza et. al., 2018; Massuquetto et. al., 2019). O aumento do consumo de ração pode ser explicado pela facilidade na apreensão do pelete, e por considerar que as aves possuem características granívora (Jensen et al., 1962). Com isso, o maior consumo de ração leva a um maior ganho de peso das aves (Massuquetto et al., 2020).

Além disso, a melhora da conversão alimentar de frangos de corte que são alimentados com dietas peletizadas pode ser melhor elucidada pela melhora da digestibilidade dos nutrientes ocasionada pelo processo físico-químico empregado no processo de peletização (Dozier et al., 2010).

Sendo assim, o aumento do consumo de ração e a melhora da digestibilidade dos nutrientes, impactam diretamente no consumo de energia. O melhor aproveitamento de carboidratos, proteínas e lipídios, acarretam melhora na disponibilidade de energia para as aves. Na literatura, diversos trabalhos mostram a melhora no conteúdo de EMA das dietas peletizadas em relação a dietas fareladas

(Serrano et. al., 2012; Jiménez-moreno et. al., 2016), porém após a EMA, a utilização da energia para manutenção e deposição de tecido apresenta perdas no processo, sendo assim, a utilização de energia pode ser melhor elucidada no sistema de EL (Kromman, 1973).

Mckinney e Teeter (2004) trabalharam com proporções de peletes e finos (100, 80, 60, 40, e 20% de peletes) concluíram que as aves que receberam maior proporção de pelete tiveram maior eficiência na utilização de energia, e atribuíram um valor de 187 Kcal EMAn/kg de ração poupada em função da forma física da ração, observando maior ganho em tecido (gordura e proteína). O menor gasto energético é considerado pelo menor tempo que a ave passa se alimentando, reduzindo sua atividade física e destinando essa energia para deposição de tecidos.

Entretanto, Maiorka et al. (2005) e Massuqueto et al. (2020) relataram que aumento da ingestão de energia, advinda pelo maior consumo de ração e pela maior disponibilidade de energia da dieta, ocasionou maior deposição de gordura no corpo, tanto em composição total quanto no conteúdo de gordura abdominal. Latshaw e Moritz (2009) ao avaliarem a partição da EM em frangos de corte, observaram maior deposição de tecidos, indicando maior conteúdo de energia líquida da dieta, e uma possível redução do incremento calórico dessas aves.

Sendo assim, o entendimento da dinâmica de utilização da energia de frangos de corte alimentados com dietas peletizadas precisa ser avaliada, e o sistema de EL se mostra o mais acurado para descrever essas respostas frente ao metabolismo energético.

4. Referências bibliográficas

Abdollahi, M. R.; Ravindran, V.; Wester, T. J.; Ravindran, G.; Thomas, D. V. Influence of feed form and conditioning temperature on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. *Animal Feed Science and Technology*, v.168, p.88-99, 2011.

Abdollahi, M.R.; Ravindran, R.; Svihus, B. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal feed science and technology* v. 179, p. 1-23, 2013.

Associação Brasileira de Proteína Animal, ABPA, 2023.

Blaxter, K. L. 1962. The Energy Metabolism of Ruminants. C. C. Thomas, Springfield, IL.

Blaxter, K. L., J. L. Clapperton, and F. W. Wainman. 1966. Utilization of energy and protein of the same diet by cattle of different ages. *J. Agric. Sci. Camb.* 67:67–73.

Brody, S. 1945. Bioenergetics and Growth. Hafner Publ. Co., New York, NY.

Brouwer, E. Report of sub-committee on constants and factors. In: Proceedings of the 3rd symposium on energy metabolism of farm animals, 1965. European association for animal production, 1965. p. 441-443.

Carré, B.; Lessire, M.; Juin, H. Prediction of the net energy value of broiler diets. *Animal* v. 8, p. 1395-1401, 2014.

Cerrate, S., Ekmay, R., England, J. A., & Coon, C. (2019). Predicting nutrient digestibility and energy value for broilers. *Poultry Science*, 98(9), 3994-4007.

Chewning, C. G., C. R. Stark, and J. Brake. Effects of particle size and feed form on broiler performance. *J. Appl. Poult. Res.* 21:830–837. 2012.

Cowieson, A. J. and Bedford, M. R. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complimentary mode of action? *World's Poultry Science Journal*, Vol. 65, 2009.

Cowieson, A. J. Factors that affect the nutritional value of maize for broilers. *Anim. Feed Sci. Technol.* 119:293–305. 2005.

Cowieson, A. J., Ruckebusch, J. P., Sorbara, J. O. B., Wilson, J. W., Guggenbuhl, P., & Roos, F. F. A systematic view on the effect of phytase on ileal amino acid digestibility in broilers. *Animal Feed Science and Technology*, 225, 182-194, 2017.

Cowieson, A. J., Toghyani, M., Kheravii, S. K., Wu, S. B., Romero, L. F., & Choct, M. A mono-component microbial protease improves performance, net energy, and digestibility of amino acids and starch, and upregulates jejunal expression of genes responsible for peptide transport in broilers fed corn/wheat-based diets supplemented with xylanase and phytase. *Poultry science*, 98(3), 1321-1332, 2019.

Cozannet, P.; Kidd, M.T.; Montanhini Neto, R.; Geraert, P.A. "Next-generation non-starch polysaccharide-degrading, multi-carbohydrase complex rich in xylanase and arabinofuranosidase to enhance broiler feed digestibility." *Poultry Science* 96: 2743-2750, 2017.

da Silva Teofilo, G. F., Lizana, R. R., de Souza Camargos, R., Leme, B. B., Morillo, F. A. H., Silva, R. L. & Sakomura, N. K. (2022). Effect of feed restriction on the maintenance energy requirement of broiler breeders. *Animal Bioscience*, 35(5), 690.

De Groote, G. (1974). A comparison of a new net energy system with the metabolisable energy system in broiler diet formulation, performance and profitability. *British Poultry Science*, 15(1), 75-95.

- Dessimoni, G. V., Sakomura, N. K., Donato, D. C. Z., Vargas, L., Melare, M., Pacheco, L., & Dalolio, F. S. (2020). Supplemental phytase derived from *E. coli* in different concentrations on performance, bone mineralization and cost of broilers diets. *Anais da Academia Brasileira de Ciências*, 92, e20180826.
- Dozier, W. A., III, K. C. Behnke, C. K. Gehring, and S. L. Branton. Effects of feed form on growth performance and processing yields of broiler chickens during a 42-day production period. *J. Appl. Poult. Res.* 19:219–226. 2010.
- Elia, M., and Livesey, G. Theory and validity of indirect calorimetry during net lipid synthesis. *American Journal of Clinical Nutrition*, 47:591-607. 1988.
- Emmans, G.C. (1994) Effective energy: a concept of energy utilization applied across species. *British Journal of Nutrition* 71: 801-821
- Farrell, D.J. (1974) General principles and assumptions of calorimetry. In: *Energy Requirements of Poultry* (Morris, T.R. and Freeman, B.M., Eds), Poultry Science Synposium No. 9, British Poultry Science Ltd, Edinburgh, p. 124
- Ferrell, C.L. e Oltjen, J.W. Asas centennial paper: Net energy systems for beef cattle - Concepts, application, and future models. *J. Anim. Sci.* 2008.
- Gautier, A. E., Walk, C. L., & Dilger, R. N. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. *Poultry Science*, 97(1), 211-218, 2018.
- Gerrits, W.J.J., Van de Borne, J.J.G.C., and Labussière, E. Deriving heat production from gaseous exchange: validity of the approach. In: *Indirect calorimetry – Techniques, computations, and applications*, edited by Gerrits, W.J.J. and Labussière, E. 2015.
- Jensen, L. S.; Merrill, L. H.; Reddy, C. V.; McGinnis, J.; Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. *Poultry Science*, v.41, p.1414–1419, 1962.
- Jiménez-Moreno, E., de Coca-Sinova, A., González-Alvarado, J. M., & Mateos, G. G. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth performance and water intake. *Poultry science*, 95(1), 41-52. 2016.
- Józefiak, D., Rutkowski, A., & Martin, S. A. (2004). Carbohydrate fermentation in the avian ceca: a review. *Animal Feed Science and Technology*, 113(1-4), 1-15.
- Kleiber, M. 1961. *The Fire of Life*. John Wiley and Sons, New York, NY.
- Kromann, R.P. 1973. Evaluation of net energy systems. *J. Anim. Sci.*
- Latshaw, J. D.; Moritz, J. S. The partitioning of metabolizable energy by broiler chickens. *Poultry Science*, v.88, p.98–105, 2009.

Le Goff, G.; Dubois, S.; Milgen, J. V.; Noblet, J. Influence of dietary fibre level on digestive and metabolic utilisation of energy in growing and finishing pigs. *Animal Research*, v.51, n.3, p.245-259, 2002.

Lehninger, T. M., Nelson, D. L. & Cox, M. M. *Princípios de Bioquímica*. 6ª Edição, 2014.

Lofgreen, G. P. and W. N. Garrett. 1968. A system for expressing net energy requirements and feed values for growing finishing beef cattle. *J. Anim. Sci.* 27: 793.

Lopez, G., & Leeson, S. (2008). Energy partitioning in broiler chickens. *Canadian Journal of Animal Science*, 88(2), 205-212.

Maiorka, A., F. Dahlke, A. M. Penz JR, and A. M. Kessler. Diets formulated on total or digestible amino acid basis with different energy levels and physical form on broiler performance. *Rev. Bras. Cienc. Avic.* 7:47–50. 2005.

Massuquetto, A., Panisson, J. C., Marx, F. O., Surek, D., Krabbe, E. L., & Maiorka, A. Effect of pelleting and different feeding programs on growth performance, carcass yield, and nutrient digestibility in broiler chickens. *Poultry science*, 98(11), 5497-5503. 2019.

Massuquetto, A., Panisson, J. C., Schramm, V. G., Surek, D., Krabbe, E. L., & Maiorka, A. Effects of feed form and energy levels on growth performance, carcass yield and nutrient digestibility in broilers. *Animal*, 14(6), 1139-1146. 2020.

McKinney, L. J., Teeter, R. G. Predicting Effective Caloric Value of Nonnutritive Factors: I. Pellet Quality and II. Prediction of Consequential Formulation Dead Zones, *Poultry Science* 83:1165 – 1174. 2004.

Mingbin, L. V., Y. Lei, W. Zhengguo, A. Sha, W. Miaomiao, and L. V. Zunzhou. Effects of feed form and feed particle size on growth performance, carcass characteristics and digestive tract development of broilers. *Anim. Nutr.* 1:252–256. 2015.

Nehring, K. 1969. Investigations on the scientific basis for the use of net energy for fattening as a measure of feed value. In K. L. Blaxter, G. Thorbek and J. Kielanowski (Ed.) *Energy Metabolism of Farm Animals*.

Noblet, J. Net energy evaluation of feed and determination of net energy requirements for pigs. *Revista Brasileira de Zootecnia*, v. 36, p. 277-284, 2007.

Noblet, J., Wu, S. B., & Choct, M. (2021). Methodologies for energy evaluation of pig and poultry feeds: A review. *Animal Nutrition*.

Noblet, J.; Dubois, S.; Lasnier, J.; Warpechowski, M.; Dimon, P.; Carré, B.; Van Milgen, J.; Labussière, E. Fasting heat production and metabolic BW in group-housed broilers. *Animal*, v. 9, p. 1138-1144, 2015.

Noblet, J.; Fortune, H.; Shi, H.; Dubois, S. Prediction of net energy value of feeds for growing pigs. *Journal of Animal Science*, p. 72, 344-354, 1994.

- Nolan, A., McDonnell, K., Devlin, G.J., Carroll, J.P., Finnan, J., 2010. Economic analysis of manufacturing costs of pellet production in the Republic of Ireland using non-woody biomass. *Open Renew. Energy J.* 3, 1–11.
- Pfeiffer, T., Schuster, S., & Bonhoeffer, S. (2001). Cooperation and competition in the evolution of ATP-producing pathways. *Science*, 292(5516), 504-507.
- Rath, N. C., Huff, G. R., Huff, W. E., & Balog, J. M. (2000). Factors regulating bone maturity and strength in poultry. *Poultry science*, 79(7), 1024-1032.
- Riveros, R. L.; Macari, M.; Reis, M. P.; Camargos, R. S.; Leme, B. B.; Sakomura, N. K. (2022). Dynamic of heat production partitioning in adult birds. *Animal Bioscience*.
- Roza, L. F., F. C. Tavernari, D. Surek, C. Sordia, L. F. T. Albino, D. Paiano, M. M. Boiago, T. G. Petrolli, and A. Cunha Júnior. Metabolizable energy and amino acid digestibility of mash and pelleted diets for broilers determined under different methodologies. *Anim. Feed Sci. Technol.* 235:1–7. 2018.
- Rutherford, S. M.; Chung, T. K.; Thomas, D. V.; Zou, M. L.; Moughan, P.J. Effect of a novel phytase on growth performance, apparent metabolizable energy, and the availability of minerals and amino acids in a low-phosphorus corn-soybean meal diet for broilers. *Poult Sci.* v.91, n. 5, p. 1118-1127, 2012.
- Sakomura, N. K., Rostagno, H. S. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: Funep, p.262, 2016.
- Schöner, F. J., and Hoppe, P.P. "The Effects of Phytase in Poultry Nutrition." In *Poultry feedstuffs: supply, composition and nutritive value*. Poultry Science Symposium Vol 26, edited by J.M. McNab and K. N. Boorman, 364-373. Wallingford, Oxon OX10 8D, UK: CABI Publishing. 2002.
- Serrano, M. P., D. G. Valencia, J. Mendez, and G. G. Mateos. Influence of feed form and source of soybean meal of the diet on growth performance of broilers from 1 to 42 days of age. 1. Floor pen study. *Poult. Sci.* 91:2838–2844. 2012.
- Sweeten, J. M. Livestock and poultry waste management: A national overview. In *National Livestock, Poultry and Aquaculture Waste Management: Proceedings of the National Workshop*. ASAE, St. Joseph, Michigan (pp. 4-14), 1992.
- Van Milgen, J.; Noblet, J.; Dubois, S. Energetic efficiency of starch, protein and lipid utilization in growing pigs. *The journal of nutrition*, v. 131, p. 1309-1318, 2001.
- Walk, C. L., & Poernama, F. Evaluation of phytase, xylanase, and protease in reduced nutrient diets fed to broilers. *Journal of Applied Poultry Research*, 28(1), 85-93, 2019.
- Walk, C. L., Bedford, M. R., Santos, T. S., Paiva, D., J. R. Bradley, J. R., H. Wladecki, H., C. Honaker, C., and McElroy, A. P. *Poultry Science* 92 :719–725, 2013.

Wu, S.; Swick, R.A.; Noblet, J.; Rodgers, N.; Cadogan, D.; and Choct, M. Net energy prediction and energy efficiency of feed for broiler chickens. Poultry Science v. 0 p. 1-13, 2018.

CAPÍTULO 2 – Effect of a novel consensus bacterial 6-phytase variant enzyme supplementation on performance, nutrient digestibility, bone mineralization, body composition, and carcass yield in broiler chickens

Effect of a novel consensus bacterial 6-phytase variant enzyme supplementation on performance, nutrient digestibility, bone mineralization, body composition, and carcass yield in broiler chickens

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INTRODUCTION

The improvement in the growth rate of modern broiler chickens due to genetic selection can result in significant differences in the optimization of nutrient utilization (Zhang et al., 2020; Kim et al., 2011). The high rate of muscle growth can create an imbalance between lean tissue deposition and skeletal growth, potentially resulting in deficiencies in bone development (Oviedo-Rondón et al., 2006). In this context, phosphorus (P) is an essential mineral for developing bone tissue, forming the skeletal structure together with calcium. Additionally, it performs other metabolic functions, such as participating in energy metabolism and developing nervous and muscular tissue as part of soft tissue (Rousseau et al., 2016).

Poultry diets, commonly based on corn and soybean meal, generally have a lower concentration of non-phytic phosphorus (NNP), since most phosphorus in vegetable sources is presented in the form of phytate. This complex inositol phosphate, a chelated structure, is considered the main form of P storage in plants (Cosgrove, 1966). Approximately 70% of the P in cereal grains and legumes is in the form of NNP, and birds have low hydrolytic power for IP₄, IP₅, and IP₆ (Morgan et al., 2015), which limits the maximum utilization of P present in the diet. The nutritional strategy employed to fulfill P requirements in commercial production is by supplementing with inorganic P sources. However, these inorganic sources are expensive. Moreover, unabsorbed phytic sources are excreted, leading to environmental pollution (Selle and Ravindran, 2007).

The negative effects associated with phytates, and reduced use of inorganic sources can be overcome, in part, by using exogenous phytases. This practice is adopted to improve the digestibility and utilization of P and reduce its antinutritional effects (Ravindran, 1995). In addition to improving phosphorus digestibility, phytases significantly reduce P excretion into

the environment (Munir and Maqsood, 2013) and increase the digestibility of other nutrients complexed with phytate (Schöner and Hoppe, 2002; Gautier et al., 2018).

The use of exogenous phytases has been evaluated in broiler chickens, showing improvements in the utilization of dietary P, bone mineralization, and performance (Cowieson and Bedford, 2009; Walk et al., 2013; Cozannet et al., 2017; Cowieson et al., 2019). Although the favorable effects of phytase supplementation on productive response have been widely evaluated, there are still gaps regarding its benefits on carcass quality and the degree of bone mineralization in diets with reduced energy and nutrients. We hypothesize that phytase supplementation improves P and other nutrient digestibility in commercial diets with reduced energy and nutrients, enhancing the bone mineralization and altering body composition, resulting in improved performance and profitability. Therefore, the aim of this paper was to evaluate the effect of phytase supplementation in diets with reduced energy, Ca, and P on the performance, nutrient digestibility, bone mineralization, body composition, and carcass yield of broiler chickens.

MATERIAL AND METHODS

The protocol was approved by Animal Care and Use Committee of the São Paulo State University (Unesp), School of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, Brazil, under protocol number n° 638/22.

Birds, experimental design, and diets

A total of 1,080 Ross 308-day-old male chicks were randomly distributed into four treatments, with nine replications of 30 birds per experimental unit (3 m x 1 m). At the start of the experiment, the birds were weighed and allocated to each experimental unit according to their body weight (40.5 ± 0.13 g). During the experimental period, the chickens had free access to water supplied by nipple-type drinkers, and the feed was offered *ad libitum* in tube feeders.

The lighting program consisted of 24 hours of continuous light from one to 14 days old, followed by an 18L:6D schedule until the end of the experiment. The trial was conducted in a negative pressure poultry house equipped with a cooling pad system to regulate temperature and humidity according to the genetic guideline (Aviagen, 2018).

The dietary treatments were formulated based on corn and soybean meal to meet the nutritional requirements recommended by Rostagno et al. (2017), except for AMEn, CP, avP, and Ca. These components were reduced to moderate and medium levels compared to the reference values. The reductions constituted control 1 (C1) and control 2 (C2), respectively, without phytase supplementation, as shown in Table 1. This reduction in nutrients and energy was in accordance with the phytase enzyme matrix contribution of 1000 and 1500 FTU/kg for their respective control treatments. The treatments with phytase supplementation were performed above each control treatment, supplementing C1 with 1000 FTU/kg and C2 with 1500 FTU/kg. During the experimental period, all treatments followed a feeding program consisting of four phases: starter (1 to 7 days old), grower I (8 to 21 days old), grower II (22 to 35 days old), and finisher (36 to 42 days old). The phytase enzyme evaluated was a novel consensus bacterial 6-phytase variant expressed in *Trichoderma reesei* (Axta PHY GOLD, Danisco Animal Nutrition & Health, IFF, Leiden, The Netherlands).

Parameters and sample collection

A representative sample of all diets was collected and stored at 4°C in plastic bags for further analysis. The samples were then taken to the laboratory for proximal analyses to determine the dry matter, crude protein, and ash content. Additionally, the phytic P content and phytase enzyme activities were determined.

At 7, 21, 35, and 42 days old, birds and feed were weighed. The variables assessed included body weight (BW) and feed intake (FI), from which the average daily gain (ADG),

average daily feed intake (ADFI), and feed conversion rate (FCR) were calculated, adjusting for daily recorded mortality.

At 21 days old, 10 birds per experimental unit were randomly selected to collect the ileal content and determine the ileal digestibility of the nutrients following the procedures described by Sakomura and Rostagno (2016). During feed manufacturing for the phase (8-21 days old), 1% of Celite® was added to the feed as an indigestible marker. Selected birds for the digestibility trial were humanely euthanized, and the celomic cavity was opened to expose and identify the third distal section of the ileum to collect the content from the final two-thirds of ileum. The ileal contents were collected in a plastic container by squeezing. Subsequently, the samples were frozen at -20°C and lyophilized at -60°C for 72 hours. After drying, they were ground in an IKA micro-grinder and homogenized for further determination of acid-insoluble ash (AIA), gross energy, crude protein, dry matter, calcium, and phosphorus content.

At 21 and 42 days old, two birds per experimental unit were randomly selected and euthanized by cervical dislocation for tibial bone collection from both legs. The tibial bones were identified, cleaned of remaining tissues, and defatted using an organic solvent (petroleum ether) for 48 hours. The bones were then dried at 105°C for six hours and ground. The processed tibias were analyzed for ash, calcium, and phosphorus composition. Tibia ash content was expressed relative to the weight of the defatted tibia, and calcium and phosphorus composition were determined relative to the ash content.

Body composition was determined through dual-energy X-ray absorptiometry (DXA) using a Hologic-QDR model 13.4.2 (Marlborough, MA). DXA scanning was performed on six birds per treatment, selected according to the average BW of the experimental unit $\pm 10\%$ tolerance. The birds were identified at 22 days old for scanning at 23 and 41 days old. The DXA scanning was performed after a fasting period of six hours. During each scanning procedure,

the birds were immobilized with an anesthetic (isoflurane diluted at 2% in pure oxygen) and positioned in a horizontal supine position with wings and legs extended. They were scanned in a craniocaudal direction (Moura, et al., 2023). The device settings included informing the BW and length of the bird to locate the scanning sensor on the table. The output of the scan included fat mass (g), lean mass (g), bone mineral content (g), and bone mineral density (g/cm²).

At 42 days old, three birds per pen were selected according to the average BW ($\pm 5\%$), subjected to a fasting period of 24 hours, and subsequently slaughtered. The birds were slaughtered using commercial processing methods, including cutting the jugular vein, chilling in hot water at 60°C to facilitate defeathering, and transferring to a table for identification and dissection of the commercial cuts. The total carcass weight was recorded, considering the defeathered carcass without feet, neck, and visceral components. The commercial cuts were divided into breast, wing, and leg (drumstick + thigh). The carcass components were expressed as relative values, as a fraction of the total carcass weight.

Proximal and chemical analysis

Dry matter content was determined by drying samples (feed, ileal digesta and bones) for 16 hours in a conventional oven at 105°C (AOAC, 2006. Method 920.39). Total nitrogen content was assessed using the Kjeldahl procedure (Foss Kjeltex 8400. AOAC Method 2001.11). The crude protein content was calculated by multiplying the nitrogen content by a factor of 6.25 (160 g N/g of protein).

Gross energy was determined by complete combustion under pressure-controlled conditions using an IKA Werke, C7000 adiabatic calorimeter (GMBH and CO., Staufen, Germany).

Calcium concentration was measured through the flame atomic absorption method using a Perkin Elmer 5000 atomic absorption spectroscope (Method no. 968.08D). Total phosphorus was quantified by the vanadate-molybdate method after ash determination at 550°C (Gomori, 1942).

Phytase activity in the feed samples was determined by Danisco Animal Nutrition & Health, IFF (São Paulo, Brazil), following a modified version of AOAC Method 2000.12 (Engelen et al., 2001).

Ash content determination in tibial bone, feed, and ileal digesta samples was conducted through total combustion in a muffle furnace at 600°C for 6 hours. Ash from feed and ileal digesta samples was subsequently subjected to insoluble acid ash determination by acid digestion with 4 mol⁻¹ HCl according to AOAC (Method 941.12). The residue remaining after acid digestion was filtered using filter paper no. 4 to separate the insoluble fraction in feed and digesta samples. The digestibility of energy and nutrients was calculated as:

$$Ndig = \frac{Ndiet - Ndigesta \times \frac{AIAdiet}{AIAdigesta}}{Ndiet}$$

Where *Ndiet* corresponds to the nutrient in the diets, *Ndigesta* is the nutrient in the digesta, *AIAdiet* is the concentration of the indigestible marker in the diets, and *AIAdigesta* is the indigestible marker in the ileal digesta.

Analysis of cost

The cost analyses were performed to evaluate the feeding cost of each treatment per kg of BWG. Based on the individual ingredient prices, the feed cost for each treatment (R\$/kg) was calculated, and the feeding cost per kg of BWG was determined. The price of each ingredient was based on the value from SindRações. The price per kg of feed was R\$1.915,

R\$1.920, R\$1.886, and R\$1.893, for C1, C1+1000FTU, C2, and C2+1500FTU, respectively. Phytase was assumed to have a value of R\$58.92/kg and was supplemented at 0.0082 and 0.0123 g/kg for 1000 and 1500 FTU respectively.

Statistical analysis

Data were subjected to variance analysis (ANOVA-one-way), and the statistical analyses were performed using the general linear model's procedures of Minitab (Minitab v19.0). When significant differences were found in ANOVA, the treatment averages were compared by pairwise contrast analyses: C1 vs. C2, C1 vs. C1 + 1000 FTU, and C2 vs. C2 + 1500 FTU. A significance level of $p < 0.05$ was considered.

$$Y_{ij} = \mu_i + \tau_j + \varepsilon_{ij}$$

Where, Y_{ij} is the response, μ_i is the overall mean effect, τ_j is the treatment effect, and the ε_{ij} is the error.

RESULTS

The analyzed composition of crude protein, calcium, total phosphorus, phytic P, and phytase activity is described in Table 1, and the values are close to those calculated for different phases and batches of manufacturing the diets.

Performance responses

The performance response variables were evaluated for each feeding phase and for the entire experimental period (1 to 42 days), as shown in Table 2. During the starter phase, significant differences ($P < 0.05$) were observed for ADG, where treatments with phytase addition at both 1000 and 1500 FTU/kg levels improved ADG compared to their respective control treatments. There was also a trend ($P = 0.054$) for improved FCR in the C2+1500FTU

treatment compared to C2. However, there were no statistical differences in broilers response for the starter phase between C1 and C2.

In the grower phases I and II (8-21 and 22-35 days, respectively), significant differences ($P<0.05$) were observed for all performance response parameters. Phytase supplementation at both levels improved ADFI ($P<0.05$) and ADG ($P<0.05$) compared to their respective control treatments. Additionally, during grower I, the control treatments differed ($P<0.05$), with C2 showing lower ADFI ($P<0.05$), ADG ($P<0.05$), and higher FCR ($P<0.05$) than C1. During the finisher phase, significant differences ($P<0.05$) were observed FCR, with C1+1000FTU having a lower FCR compared to C1. A trend ($P=0.08$) was noted for improved FCR with 1500 FTU phytase supplementation during the grower II period.

Over the entire experimental period (1 to 42 days), all performance response parameters showed statistical differences ($P<0.05$). Control treatments differed in ADG ($P<0.05$) and showed a tendency ADFI ($P=0.053$), with higher values C1 compared to C2. Phytase supplementation at both levels improved ADFI ($P<0.05$), ADG ($P<0.05$), and FCR ($P<0.05$) compared to their respective control treatments.

Digestibility of nutrients and bone mineralization

The ileal digestibility of protein, energy, calcium, and P are shown in Table 3. All digestibility parameters differed significantly between treatments ($P<0.05$). Control groups showed higher protein ($P<0.05$), Ca ($P<0.05$) and P ($P<0.05$) digestibility in C2 compared to C1. Phytase supplementation improved the digestibility of protein ($P<0.05$), energy ($P<0.05$), and P ($P<0.05$), but had no effect on calcium digestibility ($p>0.05$).

Results of tibia ash, calcium, and P content and their relationship are described in Table 4. All mineralization parameters differed significantly between treatments at both 21 and 42

days ($P<0.05$). At 21 and 42 days, the tibial ash content improved ($P<0.05$) with phytase supplementation. At 21 days, there were no differences ($p=0.424$) between control groups, but at 42 days, ash content was higher in C1 ($P<0.05$).

At 21 days, the 1500 FTU phytase supplementation improved the tibial Ca content ($P<0.05$). At 42-old-d, the 1000 FTU phytase supplementation above C1 improved tibial Ca content ($P<0.05$).

Phosphorus content in the tibia was influenced by treatments at both 21 and 42 days ($P<0.05$), with higher P content in C1 at 21 days and C2 at 42 days.

The Ca:P ratio in the tibia at 21 days showed differences for all pairwise comparisons ($P<0.05$), with C1 having a higher ratio due to higher Ca content, and phytase supplementation at 1000 and 1500 FTU reducing this ratio.

Body composition

DXA parameters scanned in birds at 23 and 41 days are shown in Table 5. At 23 days, significant effects of the experimental treatments were observed for BMC, BMD, and fat mass ($P<0.05$). Lean mass was not affected by the dietary treatments ($P>0.05$). At 23 days, C1 birds had higher BMC, BMD, and fat mass compared to C2 ($P<0.05$). Phytase supplementation at 1500 FTU improved BMC ($P<0.05$) and BMD ($P<0.05$), while 1000 FTU improved BMD ($P<0.05$) and reduced fat mass ($P<0.05$). At 41 days, BMD, fat, and lean mass effected by the experimental treatments ($P<0.05$). Phytase supplementation at 1500 FTU above the C1 diet improved BMD. Supplementation at 1000 FTU resulted in higher fat ($P<0.05$) and lean mass ($P<0.05$).

Carcass yield

The analysis of carcass physical composition is presented in Table 6. Experimental treatments influenced carcass yield ($P<0.05$) and wing weight ($P<0.05$). Phytase supplementation at 1500 FTU above C2 increased carcass yield ($P<0.05$) and reduced wings weight ($P<0.05$). Supplementation at 1000 FTU also reduced wings weight ($P<0.05$). Other responses, such as breast and leg relative weights, were not influenced by the experimental treatments.

DISCUSSION

This study was conducted to explore the effect of relative nutritional reduction of energy and nutrients on diets formulated based on the nutritional matrix of a phytase enzyme and to evaluate the beneficial effects when phytase was supplemented. The nutritional values of the nutrients and phytase activity were analyzed. Additionally, birds fed with control treatments reduced in P, Ca, lysine, and ME showed different performance responses, especially from 8 to 21 days old (Grower I). Despite the reduced nutritional values being similar between both control diets, the differences in feed composition affected the outcomes. Depending on the nutritional contribution of the ingredients, these can represent major substrates for enzyme action or ingredients with higher bioavailability of the nutritional, calcium, and phosphorus (e.g., meat and bone meal) (Liu, Cowieson, and Selle, 2016).

The relative reduction of phosphorus resulted in reduced FI, as previously reported by Viveiros et al. (2002) and Parmer et al. (1987), leading to lower BWG. The negative effects of phosphorus depletion below the requirement are well known. Aderebigbe et al (2022) mentioned that phosphorus metabolic deficiency influences appetite central control in young broiler chickens because lower blood P concentration reduces serum activation of T3, T4, and growth hormone (Parmer et al., 1987). This effect is particularly evident in phases where birds have a higher growth rate (8 to 21 days old), and consequently require a high amount of FI, as

shown in our study. Batal and Parsons (2002) reported that the effect in the first two weeks of age can be linked to greater nutrient utilization efficiency due to the rapid growth of organs and tissues.

A favorable effect of phytase supplementation at both 1000 and 1500 FTU levels was observed, improving performance responses. The phosphorus (P) released by the phytase was sufficient to counteract the negative effect of P reduction on feed intake (FI). As widely demonstrated, phytase enhances phosphorus digestibility provided there is an adequate substrate (phytates) in the diet. As shown in the digestibility trial at 21 days of age, supplementation with 1000 and 1500 FTU increased digestibility, protein utilization, and energy efficiency. This observation aligns with the findings of Selle and Ravindran (2007) and Dersjant-Li et al. (2015). The improvement in P and Ca digestibility increased the bioavailability of these nutrients and energy for birds, evidenced by better bone mineralization and reduced Ca:P ratio in the tibial bone as demonstrated by Selle and Ravindran (2007) and Selle et al. (2009), for growing chickens at 21 days old.

According to Sakomura and Rostagno (2016), bone tissue deposition should be the best parameter to prove the improvement in P digestibility in the face of a deficiency of this nutrient in diets for broiler chickens supplemented with phytase. Greater Ca and P bioavailability resulted in better bone mineralization demonstrated by higher ash content in birds fed with phytase supplementation. This effect was observed in young birds (21-days old) with a higher rate of bone tissue development and in commercial heavy birds (42-days old). This response is important for skeletal integrity to guarantee the welfare of these birds and reduce leg problems near commercial age (Oviedo-Rendon, 2008). The reduction of ash in the tibia of controls is due to P deficiency caused by the reduction of the phytase matrix, which, according to Baylink et al. (1971), reduces the rate of bone matrix formation and delays the onset of mineralization.

Furthermore, P deficiency can increase the reabsorption of this nutrient from the bone matrix (Bruin et al., 1975).

The assessment of the mineral content of tibia bones has been evaluated by several authors with phytase supplementation in P-deficient diets. However, in addition to this parameter, which describes bone mineralization in birds, recovery of bone mineralization was also found under the effect of phytase supplementation when bone mineral content and bone density were evaluated by DXA. These results showed an improvement in mineral content and bone density at 23 days old with phytase supplementation, differing from responses at 41 days old when the bone development rate is decreasing (Rousseau et al., 2016). In the finisher phase, an anorexigenic response in the control birds was not observed; the birds managed to increase their FI and consequently their P intake potentially increasing the efficiency of P utilization due to deficiency in the previous phases (Létourneau-Montminy et al., 2010). The P requirement for this phase is lower than in the starter phase, where birds are developing their entire bone structure (Driver et al., 2006).

These results further confirm that the phytate present in the diets was broken down by the action of phytase making it available for the bird to use in metabolism for bone tissue growth (Rousseau et al., 2016). Additionally, in vivo bone mineralization responses observed by DXA analysis showed responses in fat mass and lean mass content (protein + water). The lean mass content was affected at 41 days old at the level of 1000 FTU, but not at earlier stages possibly because lean mass includes both protein and water content. Phytase supplementation not only improves the P digestibility but also other nutrients and energy. In our study, lean deposition was not affected by the treatments, as the protein and digestible amino acids supply met the requirements for lean tissue synthesis. On the other hand, the additional energy available from 1000 FTU phytase supplementation was mainly deposited as fat. Although the digestible energy

for both treatments improved, birds with 1500 FTU were not affected in terms of fat deposition, likely due to higher energetic substrate in the C1 diet.

In terms of carcass yield, better results were observed for treatments that received phytase, which can be explained by improved protein digestibility and energy utilization, supporting muscle and fat tissue formation. Bone integrity plays a fundamental role in carcass quality for both whole birds and further processed (Rath et al., 2000). However, this effect was not observed for breast, thigh, and drumstick yield, unlike findings by Pillai et al. (2006), but did increase the proportion of wings in controls.

Finally, inorganic phosphorus is a costly ingredient in broiler diets, and reducing this ingredient in formulations benefits the industry by increasing profitability. In this study, a cost analysis (R\$) per kg of BWG was carried out for the total period (1-42 days), showing a gain of R\$0.06 and R\$0.08 per kg of BWG when supplementing 1000 and 1500 FTU/kg of phytase, respectively. Although inorganic phosphorus was not included as a nutrient in these formulations, there was significant gain due to the release of P from phytase. Additionally, although not measured in this study, reduced excretion of P and other nutrients into the environment can be inferred due to improved digestibility, as reported by other authors when using phytase in broiler diets (Munir and Maqsood, 2013; Kumar et al., 2015).

In conclusion, a lower energy and nutritional reduction negatively impacts feed intake and nutrient digestibility, leading to reduced mineralization and tissue syntheses. Phytase supplementation mitigates these negative effects, improving broiler responses, particularly at 1500 FTU, resulting in better productive responses and profitability.

REFERENCE

A.M. Amerah, P.W. Plumstead, L.P. Barnard, A. Kumar, Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. *Poultry Science*, Volume 93, Issue 4, 2014,

Association of Official Analytical Chemists, and Association of Official Agricultural Chemists (US). *Official methods of analysis of the Association of Official Analytical Chemists*. Vol. 2. The Association, 1925.

Baylink, D., J. Wergedal, M. Stauffer. 1971. Formation, mineralization and resorption of bone in hypophosphatemic rats. *J. Clin. Invest.* 50:2519- 2530.

Bruin, W. J., D. J. Baylink, J. E. Wergedal. 1975. Acute inhibition of mineralization and stimulation of bone resorption mediated by hypophosphatemia. *Endocrinology* 96:394- 399.

Cosgrove, D. J. (1966) The chemistry and biochemistry of inositol polyphosphates. *Review of Pure Applied Chemistry* 16: 209-215.

Cowieson, A. J. and Bedford, M. R. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complimentary mode of action? *World's Poultry Science Journal*, Vol. 65, 2009.

Cowieson, A. J., Toghyani, M., Kheravii, S. K., Wu, S. B., Romero, L. F., & Choct, M. A mono-component microbial protease improves performance, net energy, and digestibility of amino acids and starch, and upregulates jejunal expression of genes responsible for peptide transport in broilers fed corn/wheat-based diets supplemented with xylanase and phytase. *Poultry science*, 98(3), 1321-1332, 2019.

Cozannet, P.; Kidd, M.T.; Montanhini Neto, R.; Geraert, P.A. "Next-generation non-starch polysaccharide-degrading, multi-carbohydrase complex rich in xylanase and arabinofuranosidase to enhance broiler feed digestibility." *Poultry Science* 96: 2743-2750, 2017.

Dersjant-Li, Y.; Awati, A.; Schulze, H.; Partridge, G. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *Journal of the Science of Food and Agriculture*, v.95, n.5, p.878-896, 2015.

Emmans, G. C. 1981. *Animal Growth and feed intake. A collection of papers.* SAS, Edinburgh p1981-1990.

Engelen, Adrianus J. et al. Determination of phytase activity in feed by a colorimetric enzymatic method: collaborative interlaboratory study. *Journal of AOAC International*, v. 84, n. 3, p. 629-633, 2001.

Gautier, A. E., Walk, C. L., & Dilger, R. N. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. *Poultry Science*, 97(1), 211-218, 2018.

Gomori, George. "A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter." *Journal of Laboratory and Clinical Medicine* 27 (1942): 955-960.

Leme, B.B., Riveros, R.L., Camargos, R.S., Silva, R.L., Bello, A., Cunha, M.S., Dersjant-Li, Y., Sakomura, N.K. Response of broilers to supplementation of Axtra PHY GOLD phytase.2. Energy metabolism and digestibility nutrients and body composition. 2024. (dados não publicado).

Morgan, N. K., C. L. Walk, M. R. Bedford, and E. J. Burton. 2015. Contribution of intestinal- and cereal-derived phytase activity on phytate degradation in young broilers. *Poult. Sci.* 94:1577–1583.

Munir, K.; Maqsood, S. A review on role of exogenous enzyme supplementation in poultry production. *Journal of Food and Agriculture*, Abu Dhabi, v.25, n.1, p.66-80, 2013.

Parmer, T. G., L. B. Carew, F. A. Alster. 1987. Thyroid Function, Growth Hormone, and Organ Growth in Broilers Deficient in Phosphorus. *Poult. Sci.* 66:1995- 2004.

Ravindran, V. Phytases in poultry nutrition. An overview. *Poultry Science*, Champaign, v.7, p.135–139, 1995.

Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. Oliveira, D. C. Lopes, A. S. Ferreira, L. S. T. Barreto, R. F. Euclides. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Viçosa: Universidade Federal de Viçosa.

Sakomura, N. K., Rostagno, H. S. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: Funep, p.262, 2016.

Schöner, F. J., and Hoppe, P.P. "The Effects of Phytase in Poultry Nutrition." In Poultry feedstuffs: supply, composition and nutritive value. Poultry Science Symposium Vol 26, edited by J.M. McNab and K. N. Boorman, 364-373. Wallingford, Oxon OX10 8D, UK: CABI Publishing. 2002.

Selle, P.H.; Ravindran, V. Microbial phytase in poultry nutrition. Animal Feed Science and Technology, Davis, v.135, n.1, p.1- 41, 2007.

Viveros, A., A. Brenes, I. Arija, and C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci. 81:1172 -1183

Walk, C. L., Bedford, M. R., Santos, T. S., Paiva, D., J. R. Bradley, J. R., H. Wladecki, H., C. Honaker, C., and McElroy, A. P. Poultry Science 92 :719–725, 2013.

Rath, N. C., Huff, G. R., Huff, W. E., & Balog, J. M. (2000). Factors regulating bone maturity and strength in poultry. *Poultry science*, 79(7), 1024-1032.

Pillai, P. B., O'Connor-Dennie, T., Owens, C. M., & Emmert, J. L. (2006). Efficacy of an *Escherichia coli* phytase in broilers fed adequate or reduced phosphorus diets and its effect on carcass characteristics. *Poultry science*, 85(10), 1737-1745.

Rousseau, X., Valable, A.S., Létourneau-Montminy, M.P., Mème, N., Godet, E., Magnin, M., Nys, Y., Duclos, M.J., Narcy, A. Adaptive response of broilers to dietary phosphorus and calcium restrictions. Poultry Science, Volume 95, Issue 12, 2016, 2849-2860.

- Kumar, V., Singh, D., Sangwan, P., & Gill, P. K. (2015). Management of environmental phosphorus pollution using phytases: current challenges and future prospects. *Applied environmental biotechnology: present scenario and future trends*, 97-114.
- Driver, J. P., Pesti, G. M., Bakalli, R. I., & Edwards Jr, H. M. (2006). The effect of feeding calcium-and phosphorus-deficient diets to broiler chickens during the starting and growing-finishing phases on carcass quality. *Poultry Science*, 85(11), 1939-1946.
- Liu, S. Y., Cowieson, A. J., & Selle, P. H. (2016). The influence of meat-and-bone meal and exogenous phytase on growth performance, bone mineralisation and digestibility coefficients of protein (N), amino acids and starch in broiler chickens. *Animal Nutrition*, 2(2), 86-92.
- Aderibigbe, A. S., Ajuwon, K. M., & Adeola, O. (2022). Dietary phosphorus level regulates appetite through modulation of gut and hypothalamic expression of anorexigenic genes in broiler chickens. *Poultry Science*, 101(2), 101591.

Table 1. Composition and calculated nutritional value of the experimental diets

Feeding phases	Starter		Grower I		Grower I		Finisher	
Ingredients	C1 ¹	C2 ²	C1	C2	C1	C2	C1	C2
Corn	56.2	56.3	61.1	61.7	64.5	65.4	67.4	68.1
Soybean Meal 46.5%CP	37.2	37.2	33.3	33.3	30.9	30.5	28.4	28.1
Meat and Bone Meal 43%CP	2.62	2.31	1.48	1.17	0.725	0.424	-	-
Soy oil	0.352	0.301	0.993	0.740	1.82	1.50	2.14	1.83
Limestone	0.680	0.739	0.853	0.913	0.878	0.939	0.865	0.836
Sodium Bicarbonate	0.375	0.361	0.381	0.368	0.371	0.362	0.387	0.373
Sodium chloride	0.129	0.134	0.118	0.121	0.111	0.111	0.098	0.097
DL-Methionine (99%)	0.309	0.302	0.271	0.264	0.247	0.242	0.221	0.215
L-Lysine HCL (74%)	0.211	0.206	0.219	0.216	0.203	0.209	0.207	0.207
Vit - Min Premix ³	0.180	0.180	0.150	0.150	0.150	0.150	0.150	0.150
L-Threonine (98%)	0.051	0.044	0.041	0.035	0.024	0.022	0.017	0.014
L-Valine (100%)	0.034	0.029	0.030	0.025	0.025	0.025	0.022	0.019
Choline Chloride (70%)	0.050	0.050	0.050	0.050	0.050	0.050	0.050	0.050
Kaolin	1.60	1.84	-	-	-	-	-	-
Celite	-	-	1.00	1.00	-	-	-	-
Nutritional composition calculated								
ME (kcal/kg)	2894	2885	2995	2984	3106	3094	3156	3144
Crude Protein (%)	23.3 (23.2) ⁴	23.1 (23.6)	21.3 (21.3)	21.2 (21.1)	20.1 (20.5)	19.9 (19.7)	18.9 (18.2)	18.8 (19.8)
Total Calcium (%)	0.714 (0.770)	0.702 (0.760)	0.635 (0.675)	0.623 (0.630)	0.552 (0.570)	0.540 (0.570)	0.457 (0.375)	0.445 (0.405)
Available Phosphorus (%)	0.258	0.241	0.189	0.172	0.144	0.127	0.100	0.100
Total Phosphorus (%)	0.517 (0.545)	0.498 (0.530)	0.435 (0.435)	0.417 (0.410)	0.382 (0.405)	0.363 (0.410)	0.330 (0.355)	0.329 (0.345)
SID Lysine (%)	1.29	1.28	1.18	1.17	1.10	1.09	1.03	1.02
Phytic P (%)	0.245 (0.240)	0.245 (0.250)	0.239 (0.220)	0.240 (0.210)	0.236 (0.220)	0.236 (0.220)	0.232 (0.200)	0.232 (0.220)

¹ C1 control for 1000FTU of phytase.

² C2 control for 1500FTU of phytase.

³Vitamin/mineral supplied per kilogram of premix: vitamin A 11,000,000 U.I., vitamin D3 4,000,000 U.I., vitamin E 55,000 U.I., vitamin K3 3,000mg, vitamin B1 2,300 mg, vitamin B2 7,000mg, pantothenic acid 12g, vitamin B6 4,000mg, vitamin B12 25,000mcg, nicotine acid 60g, folic acid 2,000mg, biotin 250mg, selenium 300mg, iron 100g, cupper 20g, manganese 130g, zinc 130g, iodine 2,000mg.

⁴ () values analyzed.

Table 2. Performance response for broilers to starter, grower I, grower II, finisher phase, and total period to supply of phytase dose

Phase	Responses ¹	C1	C1+1000FTU	C2	C2+1500FTU	P-value	C1*C2	C1*C1+1000FTU	C2*C2+1500FTU	SEM ²
Starter	ADG (g/b/d)	13.7	14.5	13.5	15.0	<0.001	0.585	0.043	<0.001	0.146
	ADFI (g/b/d)	14.9	15.5	15.2	15.4	0.830	0.749	0.372	0.750	0.209
	FCR	1.09	1.07	1.12	1.03	0.196	0.540	0.611	0.054	0.017
Grower I	ADG (g/b/d)	47.0	51.4	43.8	50.3	<0.001	<0.001	<0.001	<0.001	0.607
	ADFI (g/b/d)	64.3	68.7	62.0	68.0	<0.001	0.023	<0.001	<0.001	0.648
	FCR	1.37	1.34	1.41	1.35	<0.001	0.002	0.017	0.002	0.006
Grower II	ADG (g/b/d)	90.9	101	90.5	103	<0.001	0.711	0.001	<0.001	1.57
	ADFI (g/b/d)	147	158	147	163	0.001	0.719	0.001	<0.001	2.17
	FCR	1.62	1.57	1.63	1.58	0.002	0.653	<0.001	0.033	0.006
Finisher	ADG (g/b/d)	117	123	112	121	0.255	0.292	0.155	0.610	1.84
	ADFI (g/b/d)	220	214	218	213	0.725	0.958	0.629	0.124	2.80
	FCR	1.88	1.74	1.95	1.76	0.027	0.131	0.017	0.080	0.011
1 to 42 days	ADG (g/b/d)	66.5	75.5	62.8	75.8	<0.001	0.006	<0.001	<0.001	1.01
	ADFI (g/b/d)	107	118	102	120	<0.001	0.053	<0.001	<0.001	1.37
	FCR	1.60	1.57	1.63	1.58	<0.001	0.128	<0.001	0.009	0.006
	R\$/kg of BWG	3.07	3.01	3.07	2.99	<0.001	0.915	<0.001	0.009	0.011

¹FCR: feed conversion ratio (g/g), ADG: average daily gain, ADFI: average daily feed intake. R\$/kgBWG revenue of chicken per kg of BWG (R\$/kg).

² SEM is pooled standard error of the mean.

Table 3. Digestibility of protein, energy, calcium, and phosphorus of broilers at 21d supply of phytase dose

Treatments	Digestible protein (%)	Digestible energy (kcal/kg)	Digestible calcium (%)	Digestible phosphorus (%)
C1	79.0	3279	75.2	67.4
C1+1000FTU	82.9	3461	77.2	83.9
C2	80.5	3327	79.9	70.4
C2+1500FTU	84.1	3485	79.4	86.1
P-value	<0.001	<0.001	0.001	<0.001
C1*C2	0.038	0.197	0.001	0.046
C1*C1+1000FTU	<0.001	0.002	0.114	<0.001
C2*C2+1500FTU	<0.001	0.001	0.653	<0.001
SEM ¹	0.407	20.9	0.493	1.45

¹ SEM is pooled standard error of the mean.

Table 4. Tibia bone mineralization and mineral composition of broilers chickens at 21- and 42 days old, consuming a feed supplemented or not with phytase

Treatments	21-old-days				42-old-days			
	Ash (g/kg)	Ca (g/kg of tibia bone ash)	P (g/kg of tibia bone ash)	Ca:P ratio	Ash (g/kg)	Ca (g/kg of tibia bone ash)	P (g/kg of tibia bone ash)	Ca:P ratio
C1	31.4	36.5	16.5	2.21	36.7	31.8	14.2	2.24
C1+1000FTU	36.9	37.1	17.3	2.14	41.6	34.9	16.1	2.17
C2	30.7	29.1	15.2	1.91	32.9	34.3	15.8	2.17
C2+1500FTU	39.1	32.1	16.4	1.96	40.8	35.1	16.7	2.10
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.006
C1*C2	0.424	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.099
C1*C1+1000FTU	<0.001	0.130	0.001	0.074	0.009	<0.001	<0.001	0.150
C2*C2+1500FTU	<0.001	<0.001	<0.001	0.183	<0.001	0.121	<0.001	0.016
SEM ¹	0.494	0.528	0.144	0.024	0.628	0.292	0.182	0.016

¹ SEM is pooled standard error of the mean.

Table 5. Body composition by DEXA of broilers at 23- and 41 days old, consuming a feed supplemented or not with phytase

Treatments	23 days				41 days			
	Bone mineral content (g/kg)	Bone mineral density (g/cm ³)	Fat (g/kg)	Lean mass (g/kg)	Bone mineral content (g/kg)	Bone mineral density (g/cm ³)	Fat (g/kg)	Lean mass (g/kg)
C1	11.2	0.085	147	850	11.3	0.113	162	827
C1+1000FTU	12.7	0.097	131	857	12.5	0.124	202	786
C2	8.76	0.071	135	865	10.6	0.104	169	820
C2+1500FTU	12.3	0.092	137	863	12.1	0.122	186	802
P-value	<0.001	<0.001	0.001	0.584	0.051	0.003	0.040	0.035
C1*C2	0.006	0.004	0.004	0.244	0.456	0.188	0.636	0.674
C1*C1+1000FTU	0.063	0.010	0.002	0.640	0.195	0.111	0.002	0.002
C2*C2+1500FTU	<0.001	<0.001	0.487	0.790	0.002	<0.001	0.373	0.337
SEM ¹	0.389	0.0024	1.64	4.04	0.268	0.0024	5.65	5.78

¹ SEM is pooled standard error of the mean.

Table 6. Carcass and cuts yield of broilers to 43 days old, consuming a feed supplemented or not with phytase

Treatments	Carcass yield (%)	Breast meat yield (% of carcass)	Leg yield (% of carcass)	Wings yield (% of carcass)
C1	76.5	37.5	28.8	10.5
C1+1000FTU	77.4	37.3	29.0	10.1
C2	76.5	37.9	29.1	10.7
C2+1500FTU	77.7	37.6	28.8	9.93
P-value	0.034	0.742	0.8670	<0.001
C1*C2	0.9350	0.4510	0.4980	0.1230
C1*C1+1000FTU	0.1090	0.7530	0.6560	0.016
C2*C2+1500FTU	0.001	0.5980	0.3320	<0.001
SEM ¹	0.180	0.184	0.140	0.056

¹ SEM is pooled standard error of the mean.

CAPÍTULO 3 – Energy utilization of broilers chickens fed with a novel consensus bacterial 6-phytase variant enzyme

Energy utilization of broilers chickens fed with a novel consensus bacterial 6-phytase variant
enzyme

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INTRODUCTION

Diets for broiler chickens are commonly based on plant-derived ingredients, particularly corn and soybean meal. A significant fraction of the phosphorus (P) in these ingredients is presented as phytate (around 60-90% of total P), which is not bioavailable to birds (Sweeten, 1992). To address this issue, exogenous phytases have been introduced into commercial poultry feed to enhance the utilization of P, other minerals, and the energy contained in the ingredients.

Phytases are phosphatases capable of hydrolyzing one or more phosphate groups from phytate complexes, thereby releasing minerals like calcium (Ca) and other minerals, as well as breaking down chelated structures into oligosaccharides, peptides, and lipoproteins. These nutrients are high-availability energy sources, efficiently used for tissue synthesis, thereby improving productive performance (Schöner and Hoppe, 2002; Gautier et al., 2018).

Animals utilize enzymes in various digestive processes to break down complex molecules, facilitating nutrient absorption and utilization. Endogenous enzymes, produced by the organism itself or by gut microorganisms, have limited catalytic power efficiency, with approximately 30% of feed remaining undigested due to anti-nutritional factors present in the ingredients (Bedford and Partridge, 2011). To mitigate this, exogenous enzymes derived from biological compounds are used in poultry feed formulation (Musigwa et al., 2020). Commercially available phytase enzymes vary in their catalytic efficiency, influenced by factors such as enzyme concentration, specific gut pH conditions required for activation, and thermal stability (Fisher et al., 2002).

The use of exogenous phytases has been extensively evaluated in broiler chicken feed, primarily focusing on improving P and Ca utilization (Cowieson and Bedford, 2009; Walk et al., 2013). However, the dynamics of energy utilization and the precise nutritional and energetic contributions of these enzymes, as determined by the net energy system, are not well defined.

The principle of using enzymes to enhance energy metabolism is based on liberating higher energy sources through improved digestibility of amino acids and simple sugars (Olukosi et al., 2008). It can be hypothesized that the metabolic pathway of these absorbed nutrients is utilized to increase protein and lipid tissue synthesis. Therefore, evaluating the benefits of enzyme use can be more accurately evidenced through net energy (NE) compared to metabolizable energy (ME) (Cowieson et al., 2018). The NE provides a better description of energy utilization by the animal, considering all energy losses (Noblet, 2007). The known positive effect of phytase utilization in commercial diets in enhancing body weight gain can be feasibly explained through the NE system (Nian et al., 2011; Barekatin et al., 2014). Therefore, this study aimed to evaluate the effect of phytase supplementation on energy utilization and to determine the net energy of feed in broiler chickens.

MATERIAL AND METHODS

The protocols were approved by the Animal Care and Use Committee of the Sao Paulo State University (Unesp), School of Agricultural and Veterinary Sciences, Jaboticabal, São Paulo, Brazil, under protocol number 5400/20.

Experimental design, animals, and facilities

A total of 160 Ross 308 male chicks, 11 days old, were used in this study. The birds were randomly distributed into four experimental diets, with five replications each. From 1 to 11 days old, the birds were raised in a conventional negative pressure poultry house with controlled ambient temperature and separated into groups, receiving experimental diets. The feed was offered in mashed form and ad libitum.

The experimental diets were formulated based on corn and soybean meal (Table 1), and consisted of: positive control (PC), meeting the nutritional requirements recommended by Rostagno et al. (2017), 3,050 kcal/kg of ME, 0.841% of Ca, 0.401% of avP, and 1.22% of AID

lysine; negative control (NC), with reduced levels of Ca (0.21%), avP (0.21%), AID lysine (0.30%), and energy (55 kcal/kg); NC plus 1000 FTU/kg of phytase; and NC plus 2000 FTU/kg of phytase.

Six open-circuit respirometric chambers (90x85x80 cm) were used to measure total heat production. They were equipped with a temperature and humidity regulation system adjusted according to the genetic guideline recommendations. The gas exchange measurements consisted of a continuous flow, with a system to sample the gas and analyze the oxygen and CO₂ concentration, performed by the Classic Sable System (Las Vegas, NV, USA). The system consists of a continuous mass flow and negative pressure pump with a subsampler, a multiplex channel alternator, a desiccant, and a set of O₂ and CO₂ gas analyzers and was calibrated as described by Riveros et al. (2023).

At 11 days old, 8 birds were selected for each experimental unit with similar average weights and moved to metabolic cages to monitor performance responses. At 14 days, the birds were transferred to the respirometric chambers for an adaptation period of two days. From 16 to 20 days old, the volume of O₂ (L) consumed and CO₂ (L) produced were recorded daily. Gas exchange measurement was recorded for 22 hours per day to calculate the total heat production and express it daily. Each day, two hours were used to collect feed remaining, provide new feed, collect excreta, and stabilize environmental gases in the respirometric chambers.

Total heat production was determined from oxygen consumption and carbon dioxide production using the Brouwer equation (1965):

$$HP (kJ)=16.17*VO_2+5.02*VCO_2$$

where HP (kJ) is the total heat production, VO₂ (L) is the volume of oxygen consumed, VCO₂ (L) is the volume of carbon dioxide produced.

During the measurement period, early in the morning, excreta were collected using the total collection method (Sakomura and Rostagno, 2016) and stored in a -20°C freezer for further analysis. Feed intake (FI) and body weight (BW) were measured daily. Diet samples were collected for analysis of dry matter, nitrogen, gross energy, Ca, total P, and phytase activity.

Apparent metabolizable energy (AME) was calculated by the difference between the gross energy intake and excreted energy. The AME was corrected by nitrogen balance, assuming 100% nitrogen retention, to obtain the apparent metabolizable energy corrected by nitrogen (AMEn), according to Sakomura and Rostagno (2016). The heat increment (HI) was obtained by the difference between the HP and the fasting heat production ($107 \text{ kcal/kg BW}^{0.75}$) determined by Noblet et al. (2015). The NE was determined by the difference between ME and HI. The body's retained energy (RE) was determined by the difference between the ME intake and HP, and the respiratory quotient (RQ) by the ratio of VCO_2 production and VO_2 consumption. The RE in the body was partitioned into RE as protein (REp), through nitrogen balance, multiplying the protein retained by the caloric coefficient of the protein (5.6 kcal/g of protein). The RE as fat (REf) was obtained by the difference in total RE and REp.

Chemical analyses

The excreta samples were thawed and homogenized, and a representative subsample was taken. These subsamples were dried in a forced ventilation oven at 55°C for 72 hours. After drying, the samples were ground in an IKA micro-grinder. The following analyses were conducted on the diet: gross energy, crude protein, and dry matter were determined, as well as the diets, Ca, total P, and phytase activity.

For dry matter content determination, samples were dried for 16 hours in a conventional oven at 105°C (AOAC, 2006. Method 920.39). Nitrogen content was determined by the Kjeldahl procedure with a Foss Kjeltac 8400 (AOAC Method 2001.11). The nitrogen value was

multiplied by 6.25 to obtain the crude protein content. Gross energy was determined using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH and CO., Staufen, Germany).

Calcium content was measured using the flame atomic absorption method on the Perkin Elmer 5000 atomic absorption spectroscope (method no. 968.08D). Total P was determined by the vanadate-molybdate method after incineration of dry samples at 550°C (Gomori, 1942). Phytase activities in feed were analyzed by Danisco Animal Nutrition & Health, IFF (São Paulo, Brazil) according to a modified version of the 2000.12 AOAC Method (Engelen et al., 2001).

Statistical analyses

Data were subjected to variance analysis (ANOVA), and the statistical analyses were performed using the general linear models' procedures of Minitab (Minitab v19.0). The means were compared using the Tukey test at 5% probability.

$$Y_{ij} = \mu_i + \tau_j + \varepsilon_{ij}$$

where the Y_{ij} represents the response, μ_i represents the overall mean effect, τ_j represents the treatment effect and the ε_{ij} is the error.

RESULTS

The diets were analyzed for dry matter, crude protein, gross energy, Ca, total P, and phytase activity. All values met the calculated formulation specifications except for the phytase activity, which remained below the calculated level.

The performance results during the experimental period in the respirometric chambers are shown in Table 2. The BWG and FI for the NC group were significantly lower ($p < 0.05$) than the other treatments. Additionally, both phytase levels did not differ ($p > 0.05$) from each other and had the same FI as the PC group. For FCR, the NC group had the highest value (1.51), but this did not differ ($p > 0.05$) from the first level of phytase (1.39). Likewise, the first level of phytase was not different ($p > 0.05$) from the PC and the second level of phytase.

The results of partitioning energy utilization in broiler chickens are presented in Table 2. The AME intake, expressed in kcal per kg of metabolic body weight, showed that chickens fed with the NC diet had a trend towards lower AME intake ($p=0.088$). However, differences were observed in the NE intake ($p<0.05$), with the PC having the highest energy intake, followed by the NC+2000 FTU group. On the other hand, the NC group had lower NE intake values, similar to the NC+1000 FTU group. The treatments did not influence the HP in the fed state and the HI of the chickens ($p>0.05$). However, the RQ was significantly lower ($p<0.05$) for the NC group compared to the other treatments. The total RE in the body ($\text{kcal/kg BW}^{0.75}$) followed the same pattern as the NE intake, with the PC group having a higher value, similar to the NC+2000 FTU group ($p<0.05$). The NC group had a lower RE, similar to the NC+1000 FTU group. When partitioning the retention into the tissue types, the RE_p differed ($p<0.05$) with birds fed the PC diet having a higher value compared to NC group. Phytase supplementation in the NC+1000 and NC+2000 FTU groups resulted in the same RE_p as the other treatments. However, the RE_f showed more variability between treatments, with the PC group having a higher RE_f, similar to the NC+2000 FTU group, followed by the NC+1000 FTU group. The NC group had a lower RE_f, similar to the NC+1000 FTU group.

The AME and AME_n values were not statistically different between treatments ($p>0.05$), indicating a relatively large variation between treatments. However, the NE determined for the feed showed a significant difference between treatments ($p<0.05$). The PC and both phytase supplementation levels (1000 and 2000 FTU) in the NC group had higher and similar values. The NC group did not differ from the phytase supplementation treatments. Additionally, the variation in terms of NE and ME was similar. The efficiency (k) for NE:AME and NE:AME_n did not differ but showed a significant trend ($p=0.083$ and $p=0.065$, respectively) towards efficiency recovery when both levels of phytase were added to the NC.

DISCUSSION

This research describes the partitioning and utilization of energy through the indirect calorimetry methodology in broilers fed low-P diets supplemented with phytase. The lower intake of AME and NE in the NC birds was due to the reduced FI and energy content of their diets. Low-P diets cause counter-responses to other nutrient and energy deficiencies, reducing poultry feed intake (Rousseau et al., 2016) and nutrient and energy digestibility (Rutherford et al., 2003; Gautier, et al., 2018; Leme et al., 2024). However, supplementing the two levels of phytase to the NC, increased the intake of AME and NE to levels close to the PC group, due to increased FI and improved energy content of the diets. Phytase can hydrolyze the phytate molecule, releasing phosphorus and other nutrients for animal metabolism (Ravindran, 1995).

Interestingly, HP was not affected by the treatments as hypothesized and suggested by Nian et al. (2011) and Barekattain et al. (2014). HP did not change between treatments since the diets had similar compositions. However, the RQ was lower for NC (0.91), indicating reduced oxidation of nutrients from carbohydrates (Gerrits et al., 2015). The addition of phytase promoted a greater release of nutrients, and the energy sources were mostly carbohydrates, increasing the RQ of treatments supplemented with phytase to the same value as the PC (0.95 and 0.96 vs 0.95), close to the value described by Gerrits et al. (2015) for carbohydrates.

Despite no differences in HP, energy intake was influenced by treatments and impacted energy retention in the body, as reported by Olukusi et al. (2008) and Cowieson et al. (2019). Birds that consumed the NC diet had lower body energy retention because of lower energy intake caused by P deficiency, lower digestibility (Leme et al., 2024), and reduced efficiency of nutrient utilization. Energy efficiency (NE:AME) showed a trend towards significance ($p=0.083$), being more efficient for PC than NC (0.71 vs. 0.74). When phytase was added, there was an increasing response of 0.72 and 0.73 for 1000 and 2000 FTU/kg, respectively. The observed improvement in energy efficiency explains the improved in FCR of birds

supplemented with phytase. Energy efficiency improves as phytase can break down complexes formed by phytic acid (Cowieson and Bedford, 2009; Gautier et al., 2018), increasing the availability of carbohydrates, proteins, and fats, thus improving digestibility and energy efficiency.

With the increase in available energy content of feed due to phytase supplementation and greater FI, energy retention as protein and fat in the body increased. The higher deposition of energy as fat is explained by the large proportion of highly digestible carbohydrates in the diets, which are easily hydrolyzed into free sugars, increasing blood glucose levels, which are then transported to the acetyl CoA pathway and transformed into triglycerides and free fatty acids (Van Milgen, 2002).

The AME and AMEn values of the diets were not significantly different between treatments ($P>0.05$), although phytase addition increased AME by 49 and 64 kcal/kg and AMEn by 39 and 56 kcal/kg for 1000 and 2000 FTU/kg of phytase, respectively. This can be attributed to the use of phytase in diets taking into account the nutritional matrix for Ca, P, amino acids, and energy. As the broiler feed formulation system is based on AME, the reduction in dietary AME, considered 55 kcal/kg in this study, was not statistically detectable. However, after energy is metabolized for maintenance or deposition as protein or fat, the energy lost as heat increment can be influenced by phytase supplementation (Kromman, 1973).

The hypothesis that phytase could efficiently release energy was better understood by comparing the effect on NE of diet treatments. Even though no statistical effect was observed between PC and NC, the improvement in energy availability was 61 and 125 kcal/kg for 1000 and 2000 FTU/kg, respectively, when phytase was added. Olukosi et al. (2008) observed a strong relationship between the body weight of broiler chickens supplemented with phytase and the NE compared to the AME system, demonstrating that the effect of phytase can be better

explained by the NE system.

In conclusion, phytase supplementation in broiler diets improves performance response and energy retention as protein and fat. The extra-phosphoric effects of phytase, such as energy release, were better understood using the NE system rather than the AME system, providing a greater supply of NE to the birds and improving energy efficiency.

REFERENCE

Association of Official Analytical Chemists, and Association of Official Agricultural Chemists (US). *Official methods of analysis of the Association of Official Analytical Chemists*. Vol. 2. The Association, 1925.

Barekatain, M.R.; Noblet, J.; Wu, S.B.; Iji, P.A.; Choct, M; Swick, R.A. Effect of sorghum distillers dried grains with solubles and microbial enzymes on metabolizable and net energy values of broiler diets. *Poultry Science*, v. 93, p. 2793-2801, 2014.

Bedford, M.R.; Partridge, G.G. *Enzymes in farm animal nutrition*. 2ed. Cabi, 2011.

Brouwer, E. Report of sub-committee on constants and factors. In: *Proceedings of the 3rd symposium on energy metabolism of farm animals*, 1965. European association for animal production, 1965. p. 441-443.

Cowieson, A. J. and Bedford, M. R. The effect of phytase and carbohydrase on ileal amino acid digestibility in monogastric diets: complimentary mode of action? *World's Poultry Science Journal*, Vol. 65, 2009.

Cowieson, A. J., M. R. Abdollahi, F. Zaefarian, G. Pappenberger, and V. Ravindran. The effect of a mono-component exogenous protease and graded concentrations of ascorbic acid on the performance, nutrient digestibility, and intestinal architecture of broiler chickens. *Anim. Feed Sci. Technol.* 235:128–137. 2018.

Cowieson, A. J., Toghyani, M., Kheravii, S. K., Wu, S. B., Romero, L. F., & Choct, M. A mono-component microbial protease improves performance, net energy, and digestibility of amino acids and starch, and upregulates jejunal expression of genes responsible for peptide

transport in broilers fed corn/wheat-based diets supplemented with xylanase and phytase. *Poultry science*, 98(3), 1321-1332, 2019.

Engelen, Adrianus J. et al. Determination of phytase activity in feed by a colorimetric enzymatic method: collaborative interlaboratory study. *Journal of AOAC International*, v. 84, n. 3, p. 629-633, 2001.

Fisher, G.; Maier, J.C.; Rutz, F.; Bermudez, V.L. Desempenho de frangos de corte alimentados com dietas a base de milho e farelo de soja, com ou sem adição de enzimas. *Revista Brasileira de Zootecnia*, v. 20, p. 402-410, 2002.

Gautier, A. E., Walk, C. L., & Dilger, R. N. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. *Poultry Science*, 97(1), 211-218, 2018.

Gerrits, W.J.J., Van de Borne, J.J.G.C., and Labussière, E. Deriving heat production from gaseous exchange: validity of the approach. In: *Indirect calorimetry – Techniques, computations, and applications*, edited by Gerrits, W.J.J. and Labussière, E. 2015.

Gomori, George. "A modification of the colorimetric phosphorus determination for use with the photoelectric colorimeter." *Journal of Laboratory and Clinical Medicine* 27 (1942): 955-960.

Kiarie, E.; Romero, L.F. and Nyachoti, C.M. "The role of added feed enzymes in promoting gut health in swine and poultry." *Nutrition research reviews* 26: 71-88, 2013.

Kromann, R.P. 1973. Evaluation of net energy systems. *J. Anim. Sci.*

Leme, B.B., Riveros, R.L., Camargos, R.S., Bello, A., Cunha, M.S., Dersjant-Li, Y., Sakomura, N.K. Effect of a novel consensus bacterial 6-phytase variant enzyme supplementation to broiler chicken diets on performance, digestibility of nutrients, mineralization, body composition, and carcass yield. 2024 (not publish).

Musigwa, S. Effect of multi-carbohydrases on energy utilisation in broiler diets. Thesis of PhD, University of New England, 2020.

- Nian, F., Guo, Y. M., Ru, Y. J., Péron, A., & Li, F. D. Effect of xylanase supplementation on the net energy for production, performance and gut microflora of broilers fed corn/soy-based diet. *Asian-Australasian Journal of Animal Sciences*, 24(9), 1282-1287. 2011.
- Noblet, J. Net energy evaluation of feed and determination of net energy requirements for pigs. *Revista Brasileira de Zootecnia*, v. 36, p. 277-284, 2007.
- Noblet, J.; Dubois, S.; Lasnier, J.; Warpechowski, M.; Dimon, P.; Carré, B.; Van Milgen, J.; Labussière, E. Fasting heat production and metabolic BW in group-housed broilers. *Animal*, v. 9, p. 1138-1144, 2015.
- Olukosi, O. A., A. J. Cowieson, and O. Adeola. Energy utilization and growth performance of broilers receiving diets supplemented with enzymes containing carbohydrase or phytase activity individually or in combination. *Br. J. Nutr.* 99:682–690. 2008.
- Ravindran, V. Phytases in poultry nutrition. An overview. *Poultry Science*, Champaign, v.7, p.135–139, 1995.
- Riveros, R., de Souza Camargos, R., Silva, R. L., Leme, B., & Sakomura, N. K. (2023). Description and Validation of Flow-Through Chambers of Respirometry for Measuring Gas Exchange in Animal Trials.
- Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. Oliveira, D. C. Lopes, A. S. Ferreira, L. S. T. Barreto, R. F. Euclides. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Viçosa: Universidade Federal de Viçosa.
- Rousseau, X., Valable, A.S., Létourneau-Montminy, M.P., Mème, N., Godet, E., Magnin, M., Nys, Y., Duclos, M.J., Narcy, A. Adaptive response of broilers to dietary phosphorus and calcium restrictions. *Poultry Science*, Volume 95, Issue 12, 2016, 2849-2860.
- Rutherford, S. M., T. K. Chung, P. C. H. Morel, P. J. Moughan. 2003. Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. *Poult. Sci.* 83:61–68.

Sakomura, N. K., Rostagno, H. S. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: Funep, p.262, 2016.

Schöner, F. J., and Hoppe, P.P. "The Effects of Phytase in Poultry Nutrition." In Poultry feedstuffs: supply, composition, and nutritive value. Poultry Science Symposium Vol 26, edited by J.M. McNab and K. N. Boorman, 364-373. Wallingford, Oxon OX10 8D, UK: CABI Publishing. 2002.

Sweeten, J. M. Livestock and poultry waste management: A national overview. In National Livestock, Poultry and Aquaculture Waste Management: Proceedings of the National Workshop. ASAE, St. Joseph, Michigan (pp. 4-14), 1992.

Van Milgen, J. (2002). Modeling biochemical aspects of energy metabolism in mammals. *The Journal of nutrition*, 132(10), 3195-3202.

Walk, C. L., & Poernama, F. Evaluation of phytase, xylanase, and protease in reduced nutrient diets fed to broilers. *Journal of Applied Poultry Research*, 28(1), 85-93, 2019.

Walk, C. L., Bedford, M. R., Santos, T. S., Paiva, D., J. R. Bradley, J. R., H. Wladecki, H., C. Honaker, C., and McElroy, A. P. *Poultry Science* 92 :719–725, 2013.

Table 1. Composition and calculated nutritional value of the positive (PC) and negative (NC) control feeds

	PC	NC
Corn	56.8	61.1
Soybean Meal 46.5% CP	34.6	33.3
Soy oil	2.89	0.993
Meat and Bone Meal 43% CP	1.45	1.48
Dicalcium Phosphate	1.15	-
Kaolin	1.00	1.00
Limestone	0.664	0.853
Sodium Bicarbonate	0.532	0.381
DL-Methionine (99%)	0.304	0.271
L-Lysine HCL (74%)	0.233	0.219
Vit - Min Premix ¹	0.150	0.150
Sodium Chloride	0.119	0.118
L-Threonine (98%)	0.070	0.041
L-Valine (100%)	0.055	0.030
Choline Chloride (70%)	0.050	0.050
Axtra PHY GOLD 10T ²	-	-
Nutritional composition calculated		
ME (kcal/kg)	3050	2995
CP (%)	21.6 (20.8)	21.3 (20.6)
Lysine (%)	1.22	1.18
Calcium (%)	0.841 (0.800)	0.635 (0.700)
Available Phosphorus (%)	0.401	0.189
Total Phosphorus (%)	0.644 (0.630)	0.435 (0.423)
Phytic P (%)	0.236	0.239

¹Vitamin/mineral supplied per kilogram of premix: vitamin A 11,000,000 U.I., vitamin D3 4,000,000 U.I., vitamin E 55,000 U.I., vitamin K3 3,000mg, vitamin B1 2,300 mg, vitamin B2 7,000mg, pantothenic acid 12g, vitamin B6 4,000mg, vitamin B12 25,000mcg, nicotine acid 60g, folic acid 2,000mg, biotin 250mg, selenium 300mg, iron 100g, cupper 20g, manganese 130g, zinc 130g, iodine 2,000mg.

²Phytase activity determined was 740 and 1360 FTU/kg for NC+1000FTU and NC+2000FTU, respectively.

Table 2. Energy partitioning and utilization of broilers chickens a control feed (PC) or a negative control feed (NC) supplemented or not with phytase (NC+1000FTU or NC+2000FTU)

Variable	PC	NC	NC+ 1000FTU	NC+ 2000FTU	P-value ⁷	SEM ⁸
Performance response						
Feed Intake (g/bird)	532 a	470 b	530 a	530 a	<0.001	6.99
Body Weight Gain (g/bird)	390 a	313 b	381 a	389 a	<0.001	7.97
Feed Conversion Ratio (g/g)	1.37 a	1.51 b	1.39 ab	1.36 a	0.011	0.019
Energy balance						
AME intake (kcal/kg ^{0.75} /d) ¹	383	363	368	380	0.088	3.28
Net Energy intake (kcal/kg ^{0.75} /d)	282 a	257 c	263 bc	278 ab	0.001	3.07
Heat Production (kcal/kg ^{0.75} /d)	210	216	214	211	0.684	1.96
Retained Energy (kcal/kg ^{0.75} /d)	173 a	147 c	154 bc	169 ab	0.001	3.09
REp (kcal/kg ^{0.75} /d) ²	81.9 a	74.3 b	78.6 ab	80.0 ab	0.031	0.995
REf (kcal/kg ^{0.75} /d) ³	90.8 a	72.3 c	75.4 bc	89.0 ab	0.003	2.45
Energy feed values						
AME (kcal/kg)	3302	3172	3221	3236	0.463	28.1
AMEn (kcal/kg) ⁴	3136	3020	3059	3076	0.489	26.1
Net Energy (kcal/kg)	2434 a	2245 b	2306 ab	2370 ab	0.023	24.4
Heat Increment (kcal/kg)	869	928	915	866	0.533	17.8
Energy utilization						
k (NE:AME) ⁵	0.74	0.71	0.72	0.73	0.083	0.005
NE:AMEn ⁶	0.78	0.74	0.75	0.77	0.065	0.005
Respiratory response						
Respiratory quotient	0.95 a	0.91 b	0.95 a	0.96 a	0.013	0.007

¹ AME intake is apparent metabolizable energy intake

² REp is energy retained as protein

³ REf is energy retained as fat

⁴ AMEn is nitrogen corrected apparent metabolizable energy

⁵ k is the ratio between net and apparent metabolizable energy

⁶ NE:AMEn is the ratio between net and nitrogen corrected apparent metabolizable energy

⁷ equal letters in a roll are statistically similar at 5% probability by Tukeys' test

⁸ SEM is pooled standard error of the mean

CAPÍTULO 4 – Effect of feed physical form on food preference, energy metabolism, performance, and carcass yield of broiler chickens

Effect of feed physical form on food preference, energy metabolism, performance, and
carcass yield of broiler chickens

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INTRODUCTION

The growth of broilers is related to their genetic potential and the efficiency of nutrient utilization. In this context, various feed and nutritional strategies have been implemented to enhance the performance of broiler chickens. Among these strategies, pelletizing stands out as a widely used feed technology in broiler diets. This process involves subjecting the feed to thermal treatment under controlled pressure and moisture conditions, generating a homogeneous pellet that corresponds to a balanced diet (Dozier et al., 2010).

Several advantages of pelletized feed over mash feed for growing chickens have been extensively evaluated, including reduced nutrient segregation (Maiorka et al., 2005), decreased time spent eating (Jensen et al., 1962), and improved nutrient values (Bolton, 1960; Roza et al., 2018). The favorable effects of pelletized feed are well-documented, highlighting increased feed intake due to the physical characteristics and density of the pellets (Roza et al., 2018). Nutrient improvements are attributed to starch gelatinization at high temperatures (around 90°C) and the disruption of disulfide bridges in polypeptide structures. This leads to protein denaturation, enhanced digestive enzyme action, and variation in the pellet passage time (Shivus, 2006). These effects result in improved nutrient digestibility, contributing to the apparent metabolizable energy (AME) value of the feed. Additionally, pelletized feed reduces energy expenditure by decreasing the frequency and duration of eating (Jensen et al., 1962; McKinney & Teeter, 2004).

The beneficial effects of pelletized feed on broiler performance have been widely reported. However, the mechanisms underlying these benefits, whether attributed to the animal or the feed, show divergent findings, particularly concerning digestibility (Roza et al., 2018; Zelenka, 2003) and the reduction in energy expended during feeding behavior of birds fed with pellets (McKinney and Teeter, 2004; Jensen et al., 1962). Conversely, many authors assert that pelletizing does not significantly improve the bioavailability of nutrients (Roza et al., 2018;

Bolton, 1960; McKinney and Teeter, 2004), or if it does, the improvement is minimal. Nevertheless, pelleting promotes an increase in body weight gain due to the increase of energy available for tissue accretion. As tissue accretion, whether fat or lean, results in increased body energy retention, this represents the fraction of net energy for production. Therefore, evaluating feed forms through energy partitioning measurements and net energy determination of feeds becomes feasible. This study aims to evaluate the impact of feed form and thermal processing on energy metabolism, as well as to elucidate their effects on carcass characteristics and performance responses in broiler chickens.

MATERIAL E METHODS

Two trials were conducted at the Poultry Sciences Laboratory (LAVINESP) of the Faculty of Agricultural and Veterinary Sciences – UNESP, Jaboticabal Campus. The study was approved by the Animal Care and Use Committee under protocol no. 5400/ 20.

Metabolism Trial

Animals, management, and experimental design

One hundred ninety-two male Cobb 500 broilers, aged 21 to 28 days, were used in the study. The birds were randomly distributed into six treatments with four replications, each consisting of eight birds. Prior to the trial, the birds were raised in a controlled environment poultry house. During the rearing and experimental periods, the diet was offered *ad libitum* in mash form, formulated according to the recommendations of Rostagno et al. (2017).

Diet preparation and experimental treatments

The experimental diets were based on corn and soybean meal, formulated to meet nutritional levels according to Rostagno et. al. (2017) as shown in Table 1. The experimental treatments were as follows: T1 – mash feed; T2 – pellet feed; T3 – crumble pellet; T4 – 50% pellet and 50% crumble pellet supplied in separate feeders; T5 – 50% pellets and 50% crumble

pellet supplied mixed in the same feeder; T6 – pair-feeding pellet in relation to the mash group.

The ingredients for the basal diets were mixed in a horizontal mixer (Model H-22, Cirelli® n. 201138, Descalvado, SP, Brazil) for five minutes. A portion of the mash feed was separated for pelletization.

The pelletizing process was conducted at the FCAV feed mill factory (Ferraz Machine, P-15) with a capacity of 250 kg/hour. The feed and moisture in the preconditioner were treated at a temperature of 85°C, and steam was injected into the barrel at a pressure of 0.5 to 1.0 kgf/cm². The treated feed was then formed into pellets using a single screener with a diameter of 2.8 mm for the first and second phases. After pellet formation, it was dried and packaged in labeled bags.

A portion of the pellets was ground using a mill equipped with a 3.8-micron screen to produce the crumble pellet treatment.

Data collection and processing

To measure heat production (HP), six open-circuit respirometric chambers (90x85x80 cm) were used, equipped with a temperature and humidity-controlled system to guarantee thermoneutrality. Continuous flow, sampling and gas analysis were performed using the Classic Sable System (Las Vegas, NV, USA). The system includes a continuous mass flow and negative pressure pump with a subsampler, a multiplex channel alternator, a desiccant, and a set of O₂ and CO₂ gas analyzers, calibrated as described by Riveros, et al. (2023).

At 17 days of age, 8 birds were selected for each experimental unit based on similar average weight and assigned their corresponding treatment. At 21 days old, the birds were transferred to the respirometric chambers for adaptation. From 22 to 26 days old, the volumes of O₂ consumed and CO₂ produced were measured for 22 hours per day to calculate the total HP. The remaining two hours were used to provide feed, collect excreta, and stabilize

environmental gases in the respirometric chambers. On day 26, the feed was removed to measure fasting heat production (FHP) for an uninterrupted period of 24 hours. Heat production (HP) was determined from O₂ consumption and CO₂ production, using the Brouwer equation (1965):

$$HP(kJ)=16.17*VO_2+5.02*VCO_2$$

where HP (kJ) is the total heat production; VO₂ (L) is the volume of oxygen consumed; VCO₂ (L) and the volume of carbon dioxide produced.

During the HP data collection period, excreta were collected using the total collection method (Sakomura and Rostagno, 2016), and feed intake was monitored, including feed leftovers to determine the actual feed intake of pellets and fine particles separately. Bird weight was also measured to express HP per unit of metabolic body weight (kg^{0.75}). The collected excreta were homogenized and analyzed for dry matter, crude protein, and gross energy content to calculate AME and AMEn.

At the end of the trial, the visceral mass of two birds per chamber was analyzed. The free-feather body weight and the weight of the gut tract components, including proventriculus, gizzard, gut, liver, and abdominal fat, were measured.

Calculations

Metabolizable energy was calculated using gross energy intake minus excretion, and apparent metabolizable energy corrected by nitrogen balance according to Sakomura and Rostagno (2016). The HI was obtained by the difference between the HP and the FHP, (85 kcal/kg BW^{0.75}), determined by the average of all treatments. Net energy (NE) was determined by the difference between ME minus HI. Additionally, the NE/ME and NE/AMEn ratios were calculated to express the efficiency of energy utilization. The energy retained in the body was calculated by the difference between ME intake and HP. The energy retained in the body was

partitioned into energy retained as protein, through nitrogen balance, multiplying the protein retained by the caloric coefficient of the protein (5.6 kcal/kg of protein), and energy retained as fat, by the difference in total energy retained and energy retained as protein. The respiratory quotient (RQ) was determined by the ratio of CO₂ consumption and O₂ production.

Performance Trial

A trial was conducted in an experimental poultry house to describe performance, feeding behavior and preference, carcass yield and body composition of broiler chickens. The treatments used in this trial were the same as those in the metabolism trial, except for the pair-feeding group treatment, which was not feasible to perform in this trial.

Experimental design and animals

A total of 1500 one-day-old male Cobb 500 chicks were used and raised in an environmentally controlled poultry house. From one to 11 days old, the birds were fed a mash diet that met the requirements recommended by Rostagno et al. (2017). At 11 days-old, the birds were randomly allocated to five treatments of 10 replications of 30 birds each, ensuring similar weight across experimental units. The experimental diets were the same as those used in the metabolism trial and were divided into two phases: 11 to 21 days and 22 to 42 days.

Data collection

At 21 and 42 days of age, the birds were weighed per pen, and the residual feed was measured to calculate feed intake. As in the previous trial, the feed leftovers from treatments 4 and 5 were weighed, separating the pellets and fine particles using a screener to calculate the actual intake of pellets. Feed conversion ratio was calculated based on BWG and FI, corrected for mortality.

At the end of the trial, at 42 days old, three birds of average weight from each pen were selected and fasted for 24 hours. After fasting, they were slaughtered to evaluate the yield of

carcass, breast, thigh and drumstick, and abdominal fat. Additionally, body composition was determined using the comparative slaughter method. Six birds were slaughtered at 11 days old at beginning of the experiment, and one bird of average weight per pen was slaughtered at the end of the trial. Body composition was analyzed to calculate the deposition of fat and protein in the body.

Chemical analysis

Diet samples were collected for analysis of dry matter, nitrogen, and gross energy. The excreta were thawed, homogenized and a representative subsample was taken and dried in a forced ventilation oven at 55°C for 72 hours. After pre-drying, the samples were ground in an IKA micro-grinder and analyzed for gross energy, nitrogen, and dry matter. Feather and body samples from the performance trial were collected and analyzed separately. The feathers were pre-dried in a forced ventilation oven at 55°C for 72 hours, then ground to determine the dry matter and nitrogen content. The body was sawed and ground in a meat mill, homogenized, and an aliquot was removed in a frozen petri dish and dried in a freeze dryer at -60°C for 72 hours. After the first drying, the samples were ground in an IKA micro-grinder and analyzed for dry matter, nitrogen, and fat content.

For dry matter content, the samples were dried for 16 hours in a conventional oven at 105°C (AOAC, 2006. Method 920.39). Nitrogen was determined by the Kjeldahl procedure (Foss Kjeltex 8400. AOAC Method 2001.11) and multiplied by 6.25 to obtain the crude protein content. Gross energy was measured using an adiabatic bomb calorimeter (IKA Werke, C7000, GMBH and CO., Staufen, Germany). Fat was measured using the AnkomXT15 extractor (ANKOM Technology, Macedon, NY) with petroleum ether solvent (AOAC method 920.39).

Statistical analysis

The data were subjected to analysis of variance (ANOVA-One-way). When significant

differences at the 95% confidence interval were obtained, the mean comparison test was performed by Tukey at a 5% probability level. Additionally, contrast analyses were made between treatments 1 vs. 2 vs. 3 (mash, pellets, and crumble pellet), 4 vs. 5 (50% pellets and 50% crumble pellet supplied in separate feeders; and 50% pellets and 50% crumble pellet supplied mixed), 1 vs. 6 (crumb and pair feeding). Statistical analyses were performed using Minitab v.20 statistical software (Minitab Inc., State College, PA, USA).

RESULTS

Feed processing

The pelletizing quality of the feed was evaluated using pellet durability index (PDI) according to Ensminger (1985), obtaining values of 96.6% and 93.1% for the phases from 11 to 21 days old and 22 to 42 days old, respectively. A sample of the pellet diets was evaluated for friability using a friability tester, subjecting the pellets to rotation and screening to determine fines generation. The average value was expressed as the pellet to grounded pellet ratio, which was 90.6:9.4 and 88.4:11.6 for the first and second phases, respectively. Additionally, the pellet diets were evaluated for size and shape using a caliper, obtaining an average size of 6.12 ± 1.8 cm and 7.81 ± 1.4 cm for the first and second phases, respectively, with a diameter of 2.8 mm.

Energy metabolism

The initial body weight of the birds was similar (559 ± 20 g) across treatments. During the evaluation, period, a significant difference in final BW was observed between treatments ($p < 0.05$). Birds that received pelleted feed had a higher final weight ($p < 0.05$) compared to those that received mashed and crumble pellet feed. Additionally, birds that received crumble pellet feed had a lower final weight ($p < 0.05$) than those in the mash group. Feed intake was higher ($p < 0.05$) for the pelleted feed treatment and did not differ between mash and crumble pellet feed ($p > 0.05$). When comparing pellets and crumble pellets supplied in separate versus mixed

feeders, birds that received mixed diets had higher ($p<0.05$) final weight and feed intake, showing a greater preference for pellet intake.

Energy partitioning

The energy partitioning results are shown in Table 3. No significant effect ($p>0.05$) was observed for AME and AMEn, although there was a trend towards significance (0.064 and 0.059, respectively). Comparing the mash, pellet and crumble pellet treatments, there was significant difference ($p<0.05$) in AME and AMEn content, with higher values for the pelleted feed. No effect was observed ($p>0.05$) for AME and AMEn between pellets and grounded pellets. For NE values, there was a significant effect ($p<0.05$). Among the mash, pellet, and crumble pellet treatments, there was only a trend towards significance ($p=0.092$), with the NE value of the pelleted feed being higher than that of crumble pellet treatment. The efficiencies of utilization of GE to AME and AMEn, as well as AME and AMEn to NE, were not significantly different ($p>0.05$), but the pelleted diets presented an efficiency 5% higher than the mashed diet. The birds' intake of AME and NE showed differences ($p<0.05$), being lower for mashed feed and fines, following the same pattern as feed intake. The mashed feed presented numerically higher HP (170 vs. 161 kcal/kg^{0.75}) and HI (85.5 vs. 76.4 kcal/kg^{0.75}), than the pelleted feeds, and the RQ did not differ between treatments. Birds that received mashed and grounded pellet diets had lower energy retention in the body ($p<0.05$). The pair-feeding group receiving the mashed feed showed lower energy retention as protein compared to others that were similar. Fat retention was lower ($p<0.05$) for birds that received mashed and grounded pellet diets.

Organ weights

The weights of the gizzard, proventriculus, liver, gut, and abdominal fat are presented in Table 4. The relative weight of the gizzard was higher ($p<0.05$) in birds that received a mashed

diet compared to those that received a pelleted diet. Conversely, the relative weights of the proventriculus, liver, and gut were greater ($p<0.05$) in birds that received a pelleted diet (pellet or fines). The relative weight of abdominal fat was higher ($p<0.05$) in birds fed pellets.

Performance

The performance results of birds from 11 to 42 days-old are presented in Table 5. For the first phase, from 11 to 21 days old, a significant effect ($P<0.05$) was observed for FI, BWG, and FCR. FI was significantly lower ($p<0.05$) in birds that received a mashed diet and was similar among the others groups. The same trend was observed for BWG. However, for FCR, birds that received pellets, or a mixture of pellets and fines (in separate feeders or mixed), presented higher efficiency ($p<0.05$) converting feed into weight gain. In the second phase, from 22 to 42 days old, significant effects were observed only for FI and BWG ($p<0.05$), following the same trend as the previous phase. Over the entire period from 11 to 42 days old, significant effects were observed for FI and BWG ($p<0.05$), with a trend towards significance for FCR ($P=0.083$), showing improved FCR for pelleted diets (pellets and crumble pellet).

Feed Behavior and preference

The results of feed behavior and preference for treatments containing pellets and crumble pellets, in separate or mixed feeders, are presented in Table 6. The pellet to crumble pellet ratio in the first week did not differ significantly ($p=0.055$) between the two groups but showed a tendency for the group of birds that received pellets and grounded pellet mixed in the same feeder. Over the next three weeks, the treatment in which pellets and fines were provided in separate feeders showed a greater preference ($p<0.05$) for pellets. When pellets and fines were offered mixed, the intake remained at 50% pellets and 50% fines.

Carcass Yield and body composition

The results for carcass yield, cuts, abdominal fat, and protein and fat deposition in the

body are shown in table 7. No significant effect of treatments was observed on carcass, breast, or leg yield ($p>0.05$). For wing yield, treatments that received pellets and pellets to crumble pellets presented lower values ($p<0.05$) compared to birds that received a mashed diet and fines. Birds that received pellets and pellets to crumble pellets had greater abdominal fat deposition ($p<0.05$) than birds that received mashed or fines diets.

DISCUSSION

The present study aims to explain the effects of different physical forms and processing methods of feed presented as pellet, mash, and crumble pellet diets on the energy partitioning and utilization by broiler chickens, and to investigate the effect of feed form on broilers responses. Two trials were conducted to assess energy metabolism parameters and practical performance responses. Additionally, feeding behavior was evaluated by measuring actual pellet intake compared to crumble pellet particles in single and separated feeders.

The higher FI with pellet feed observed in this study confirms the findings of Abdollahi et al. (2011), Chewning et al. (2012), Abdollahi et al. (2013), Mingbin et al. (2015), Roza et al. (2018), and Massuquetto et al. (2019). In metabolism trial, pellet intake was 17% higher than mash feed, the highest value relative to existing literature, such as Massuquetto et al. (2020) who reported an 11% increase. This may be due to the short experimental period, with five days for diet adaptation and four days for data collection. Similarly, Roza et al. (2018) observed a 15.3% increase in the pellet intake in 18-to-22-day-old birds.

The age of the birds also influenced FI. In performance trial, during the first phase (11 to 21 days), the increase in pelleted intake compared to mash feed was 8.9%. Lopez et al. (2007) found a 7.5% increase in FI for broiler chickens receiving pelleted diets from 1 to 45 days and Chewning et al. (2012) observed an 8.4% increase from 1 to 21 days. In this study, during the second phase (22 to 42 days), the FI difference between broilers consuming a pellet and mash feeds decreased to 5.7%, likely due to increased FI with age, reducing the effect of greater feed

voracity seen in younger birds.

Another factor affecting FI in this study was pellet quality. The pellet durability index (PDI) was above 80%, considered good quality. McKinney and Teeter (2004) observed that birds reduced FI as the proportion of crumble pellet increased. In metabolism trial, FI with crumbled pellet feed was lower than with pellet feed (29%) and mash feed (11%). However, in performance trial, with a longer adaptation period, intake of fine particles was lower than pellet feed but higher than mash feed.

When evaluating birds' food preferences, no differences in FI were observed in performance trial but there were differences in metabolism trial. This can be attributed to the evaluation period, as birds show a strong preference for large particle size feed. Additionally, the type of feeder used affected the birds' responses. In metabolism trial, a trough-type feeder allowed birds to choose their feed, resulting in higher FI for mixed feed (pellet:fine), with a preference for pellets. In performance trial, a tubular feeder limited feed choice, resulting in equal intake of pellets and fines.

Although the main effect on broiler chickens receiving pelleted diets is on FI, nutrient digestibility also improved. Abdollahi et al. (2010) and Zhu et al. (2016) reported improved nutrient digestibility in pelleted feed due to the pelletization process, which adds water vapor at pressure, partially gelatinizing starch and denaturing protein structures, facilitating enzyme action (Dozier et al., 2010). This improved nutrient digestibility is evidenced by better energy utilization, as energy in feed results from the oxidation of digested and absorbed nutrients (Lehninger et al., 2014). In metabolism trial, there was a tendency for improved AME and AMEn content in pelleted feed compared to mash feed. However, the difference between pelleted and fine feed may be due to increased energy expenditure during food capture (McKinney and Teeter, 2004). This is further supported by the NE content difference between

pellet and fine feed (189kcal/kg). similar to the energy saved by utilization of pelleted feed (187kcal/kg), as reported by McKinney and Teeter (2004).

The feed pelleting process improves nutrient availability, but has less effect compared to more intense processes like extrusion and expansion (Svihus et al., 2005). Although no statistical effect on energy utilization was observed, pelleted diets were around 5% more efficient than mash diets, due to improved nutrient metabolism. Differences in FI and energy content of the feed impacted ME and NE intake, influencing energy retention in the body. No statistical effect between mash and pelleted diets was observed for HP, but the mash diet had the highest HP due to facilitated nutrient digestion in pelleted feed, generating lower HP (Dozier et al., 2010). The RQ was unaffected by the treatments with an average value of 1.03 indicating higher carbohydrate oxidation (Gerrits et al., 2015).

Energy retention was higher in birds fed pelleted diets, mainly as fat confirmed by a higher percentage of abdominal fat, in both trials. Higher FI led to excess energy intake used for maintenance and growth, with the remainder stored as adipose tissue (Massuqueto et al., 2020). Other studies also found increased abdominal fat deposition in broilers fed pelleted diets (Maiorka et al., 2005 and Corzo et al., 2011). The higher FI and energy intake may explain the improved performance of birds fed pelleted feed in performance trial, particularly in the first phase (11 to 21 days), which showed better FCR for birds on the pellet diet.

The weight of the GIT and liver changed with pelleted feed compared to mash feed. The relative weight of the gizzard was lower in birds fed pellets, likely due reduced coarser particles and less muscle activity needed to grind them (Santos et al., 2008; Svihus et al., 2014). Conversely, the relative weight of the proventriculus, liver, and gut was higher in birds fed pelleted feed, due to increased FI and nutrient metabolization. No effects were observed on carcass, breast, or leg yield, consistent with findings by Mingbin et al. (2015) and Massuqueto

et al. (2020), indicating that feed does not affect carcass yield. However, wing yield was lower in birds fed pellet, as the carcass was larger in absolute weight while wing weight remained unchanged.

In conclusion, broiler chickens fed pelleted feed had higher FI, greater BWG, and improved FCR. Birds showed a preference for pellets but required an adaptation period. Pelleted feed also resulted in higher NE utilization and greater energy retention in the body, primarily as fat, suggesting that the NE system better represents energy utilization based on feed form.

REFERENCE

- Brouwer, E. Report of sub-committee on constants and factors. In: Proceedings of the 3rd symposium on energy metabolism of farm animals, 1965. European association for animal production, 1965. p. 441-443.
- Noblet, J.; Dubois, S.; Lasnier, J.; Warpechowski, M.; Dimon, P.; Carré, B.; Van Milgen, J.; Labussière, E. Fasting heat production and metabolic BW in group-housed broilers. *Animal*, v. 9, p. 1138-1144, 2015.
- Riveros, R., de Souza Camargos, R., Silva, R. L., Leme, B., & Sakomura, N. K. (2023). Description and Validation of Flow-Through Chambers of Respirometry for Measuring Gas Exchange in Animal Trials.
- Rostagno, H. S., L. F. T. Albino, J. L. Donzele, P. C. Gomes, R. F. Oliveira, D. C. Lopes, A. S. Ferreira, L. S. T. Barreto, R. F. Euclides. 2017. Tabelas brasileiras para aves e suínos: composição de alimentos e exigências nutricionais. 4.ed. Viçosa: Universidade Federal de Viçosa.
- Sakomura, N. K., Rostagno, H. S. Métodos de pesquisa em nutrição de monogástricos. Jaboticabal: Funep, p.262, 2016.

Dozier, W. A., III, K. C. Behnke, C. K. Gehring, and S. L. Branton. Effects of feed form on growth performance and processing yields of broiler chickens during a 42-day production period. *J. Appl. Poult. Res.* 19:219–226. 2010.

Massuquetto, A., Panisson, J. C., Marx, F. O., Surek, D., Krabbe, E. L., & Maiorka, A. Effect of pelleting and different feeding programs on growth performance, carcass yield, and nutrient digestibility in broiler chickens. *Poultry science*, 98(11), 5497-5503. 2019.

Massuquetto, A., Panisson, J. C., Schramm, V. G., Surek, D., Krabbe, E. L., & Maiorka, A. Effects of feed form and energy levels on growth performance, carcass yield and nutrient digestibility in broilers. *Animal*, 14(6), 1139-1146. 2020.

McKinney, L. J., Teeter, R. G. Predicting Effective Caloric Value of Nonnutritive Factors: I. Pellet Quality and II. Prediction of Consequential Formulation Dead Zones, *Poultry Science* 83:1165 – 1174. 2004.

Serrano, M. P., D. G. Valencia, J. Mendez, and G. G. Mateos. Influence of feed form and source of soybean meal of the diet on growth performance of broilers from 1 to 42 days of age. 1. Floor pen study. *Poult. Sci.* 91:2838–2844. 2012.

Svihus, B., Uhlen, A.K., Harstad, O.M. Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Anim. Feed Sci. Technol.* 122, 303–320. 2005.

Jiménez-Moreno, E., de Coca-Sinova, A., González-Alvarado, J. M., & Mateos, G. G. Inclusion of insoluble fiber sources in mash or pellet diets for young broilers. 1. Effects on growth performance and water intake. *Poultry science*, 95(1), 41-52. 2016.

Amerah, A. M., V. Ravindran, R. G. Lentle, and D. G. Thomas. Feed particle size: Implications on the digestion and performance of poultry. *World's Poult. Sci. J.* 63:439–455. 2007^a.

Jensen, L. S.; Merrill, L. H.; Reddy, C. V.; McGinnis, J.; Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. *Poultry Science*, v.41, p.1414–1419, 1962.

Abdollahi, M. R.; Ravindran, V.; Wester, T. J.; Ravindran, G.; Thomas, D. V. Influence of feed form and conditioning temperature on performance, apparent metabolisable energy and ileal digestibility of starch and nitrogen in broiler starters fed wheat-based diet. *Animal Feed Science and Technology*, v.168, p.88-99, 2011.

Abdollahi, M.R.; Ravindran, R.; Svihus, B. Pelleting of broiler diets: An overview with emphasis on pellet quality and nutritional value. *Animal feed science and technology* v. 179, p. 1-23, 2013.

Chewning, C. G., C. R. Stark, and J. Brake. Effects of particle size and feed form on broiler performance. *J. Appl. Poult. Res.* 21:830–837. 2012.

Mingbin, L. V., Y. Lei, W. Zhengguo, A. Sha, W. Miaomiao, and L. V. Zunzhou. Effects of feed form and feed particle size on growth performance, carcass characteristics and digestive tract development of broilers. *Anim. Nutr.* 1:252–256. 2015.

Roza, L. F., F. C. Tavernari, D. Surek, C. Sordia, L. F. T. Albino, D. Paiano, M. M. Boiago, T. G. Petrolli, and A. Cunha J´unior. Metabolizable energy and amino acid digestibility of mash and pelleted diets for broilers determined under different methodologies. *Anim. Feed Sci. Technol.* 235:1–7. 2018.

Bolton, W. The digestibility of mash and pellets by chicks. *Journal Agriculture Science*, v.55, p.141-142, 1960.

Corzo, A., L. Mejia, and I. I. R. E. Loar. Effect of pellet quality on various broiler production parameters. *J. Appl. Poult. Res.* 20:68–74. 2011.

Latshaw, J. D.; Moritz, J. S. The partitioning of metabolizable energy by broiler chickens. *Poultry Science*, v.88, p.98–105, 2009.

Maiorka, A., F. Dahlke, A. M. Penz JR, and A. M. Kessler. Diets formulated on total or digestible amino acid basis with different energy levels and physical form on broiler performance. *Rev. Bras. Cienc. Avic.* 7:47–50. 2005.

Association of Official Analytical Chemists, and Association of Official Agricultural Chemists (US). *Official methods of analysis of the Association of Official Analytical Chemists*. Vol. 2. The Association, 1925.

López, C. A. A.; Baião, N. C.; Lara, L. J. C.; Rodriguez, N. M.; Cançado, S. V. Efeitos da forma física da ração sobre a digestibilidade dos nutrientes e desempenho de frangos de corte. *Arquivos Brasileiros de Medicina Veterinária e Zootecnia*, v.59, p.1006-1013, 2007.

Abdollahi, M. R.; Ravindran, V.; Wester, T. J.; Ravindran, G.; Thomas, D. V. Influence of conditioning temperature on performance, apparent metabolisable energy, ileal digestibility of starch and nitrogen and the quality of pellets, in broiler starters fed-maize and sorghum-based diets. *Animal Feed Science and Technology*, v.162, p.106–115, 2010.

Zhu, L.; Jones, C.; Guo, Q.; Lewis, L.; Stark, C. R.; Alavi, S. An evaluation of total starch and starch gelatinization methodologies in pelleted animal feed. *Journal of Animal Science*, v.94(4), p. 1501-1507, 2016.

Table 1. Ingredients and nutritional composition of experimental diets

Ingredients (%)	11-21d	22-42d
Corn (7.88%)	53.8	57.2
Soybean meal (45%)	37.9	34.8
Soybean oil	3.90	4.00
Limestone	0.880	0.840
Dicalcium phosphate	1.77	1.53
Sodium Chloride	0.520	0.500
DL Metionine (xx%)	0.390	0.360
Lysine HCL (xx%)	0.250	0.250
L treonine (xx%)	0.130	0.120
L valine (xx%)	0.100	0.100
Vit. and Min premix ¹	0.160	0.160
Choline chloride (70%)	0.050	0.050
Salinomycin	0.100	0.100
Nutritional composition calculate (%)		
Metabolizable Energy (kcal/kg)	3050	3100
Crude Protein	22.06	20.87
SID Lysine	1.31	1.24
SID Met+Cys	0.966	0.914
SID Treonine	0.862	0.815
SID Valine	1.01	0.951
SID Triptophan	0.248	0.233
Calcium (%)	0.907	0.822
Available Phosphorus (%)	0.432	0.384
Sodium (%)	0.221	0.211

¹Vitamin and mineral supplied per kilogram of premix: vitamin A 11,000,000 U.I., vitamin D3 4,000,000 U.I., vitamin E 55,000 U.I., vitamin K3 3,000mg, vitamin B1 2,300 mg, vitamin B2 7,000mg, pantothenic acid 12g, vitamin B6 4,000mg, vitamin B12 25,000mcg, nicotine acid 60g, folic acid 2,000mg, biotin 250mg, selenium 300mg, iron 100g, cupper 20g, manganese 130g, zinc 130g, iodine 2,000mg.

Table 2. Final body weight (BW), fee intake (FI), and food preference of broiler chickens 21 to 26 days-old in the energy metabolism trial

	Final BW (kg/ave)	FI (kg/ave)	Food preference (pellet:crumble)
Mash	1.284	0.484	-
Pellet	1.436	0.567	-
Crumble	1.071	0.438	-
P+C ¹ (separate)	1.229	0.492	1.028:1
P+C (mix)	1.339	0.566	3.038:1
Pellet <i>pair feeding</i>	1.220	0.484	-
p-value	<0.0001	<0.0001	-
SEM ²	0.025	0.012	0.520
1 vs. 2 vs. 3 ³	<0.0001	0.003	-
4 vs. 5 ⁴	0.068	0.043	0.040
1 vs. 6 ⁵	0.054	0.980	-

¹P+C, pellet plus crumble. ²SEM is pooled standard error of the mean. ³Interaction between feed processing, 1 – Mash; 2 – Pellet; 3 – crumble. ⁴Comparison between the form of supply of pellet and fine feed. ⁵Interaction between the *pair feeding* group (pellet) and mash.

Table 3. Energy partitioning in broiler chickens fed a mash, pellet, and crumble pellet diets from 21 to 26 days-old (trial 1)

Variables	Mash	Pellet	Crumble	P+C ¹ (separate)	P+C (mix)	Pellet pair feeding	P-value	SEM	1*2*3 ²	4*5 ³	1*6 ⁴
Energy balance											
MEi (kcal/kg ^{0.75})	349	365	338	360	391	345	0.004	4.68	0.099	0.072	0.589
NEi (kcal/kg ^{0.75})	263	287	261	288	312	277	0.012	4.95	0.129	0.147	0.274
HP (kcal/kg ^{0.75})	170	162	163	157	163	152	0.203	2.09	0.441	<0.0001	0.119
HI (kcal/kg ^{0.75})	85.5	77.4	77.5	71.8	78.9	67.4	0.191	2.10	0.432	<0.0001	0.115
RE (kcal/kg ^{0.75})	179	203	175	203	228	193	0.011	4.97	0.115	0.142	0.282
REp (kcal/kg ^{0.75})	79.2	79.4	78.3	78.5	87.0	73.3	0.031	1.22	0.964	0.030	0.020
REf (kcal/kg ^{0.75})	99.5	124	97.0	124	141	119	0.006	4.17	0.043	0.229	0.123
Energy feed values											
ME (kcal/kg)	3383	3474	3255	3426	3498	3344	0.064	25.1	0.032	0.061	0.700
AMEn (kcal/kg)	3202	3301	3080	3250	3315	3177	0.059	24.3	0.030	0.067	0.789
NE (kcal/kg)	2552	2676	2487	2739	2781	2690	0.023	29.3	0.092	0.202	0.270
Energy utilization											
k (ME:GE)	0.73	0.74	0.71	0.74	0.75	0.73	0.085	0.005	0.165	0.061	0.911
AMEn:GE	0.69	0.70	0.67	0.70	0.72	0.69	0.085	0.004	0.142	0.067	0.826
k (NE:ME)	0.75	0.79	0.77	0.80	0.80	0.80	0.143	0.006	0.305	0.843	0.127
NE:AMEn	0.80	0.83	0.81	0.84	0.84	0.85	0.204	0.007	0.367	0.909	0.148
Respiratory response											
RQ	1.022	1.025	1.033	1.048	1.050	1.036	0.077	0.003	0.359	0.106	0.056

¹P+C, pellet plus crumble. ² Interaction between feed processing, 1 – Mash; 2 – Pellet; 3 – Crumble. ³ Comparison between the form of supply of pellet and crumble feed. ⁴ Interaction between the *pair feeding* group (pellet) and mash.

Table 4. The relative weight of organs of broiler chickens fed with mash, pellet, and crumble diets, from 21 to 26 days-old (trial 1)

	Gizzard	Proventriculus	Liver	Gut	Abdominal fat
Mash	2.74	0.590	4.11	4.61	0.572
Pellet	2.21	0.780	4.87	5.84	0.859
Crumble	2.52	0.800	5.09	7.18	0.441
P+C ¹ (separate)	2.58	0.750	5.33	5.98	0.611
P+C (Mix)	2.71	0.780	5.77	5.79	0.854
Pellet pair feeding	2.20	0.690	5.09	6.14	0.880
P-value	0.009	0.115	0.028	0.010	0.002
SEM	0.058	0.025	0.146	0.201	0.042
Contrasts					
1 vs. 2 vs. 3 ²	0.007	0.060	0.006	0.007	0.008
4 vs. 5 ³	0.576	0.720	0.538	0.674	0.017
1 vs. 6 ⁴	0.003	0.190	<0.0001	0.008	0.042

¹P+C, pellet plus crumble. ² Interaction between feed processing, 1 – Mash; 2 – Pellet; 3 – Crumble. ³ Comparison between the form of supply of pellet and crumble feed. ⁴ Interaction between the pair feeding group (pellet) and mash.

Table 5. Performance of broiler chickens fed mash, pellet, **crumble** and mixture of pellets and **crumble** diets, from 11 to 21, 22 to 42 and 11 to 42 days-old (trial 2)

	Variables	Mash	Pellet	Crumble	P+C ¹ (separate)	P+C (mix)	p-value	SEM	1*2*3 ²	4*5 ³
11-21 d	FI (g/b/d)	79.6	86.7	85.5	86.4	86.3	<0.0001	5.55	<0.0001	0.988
	BWG (g/b/dia)	60.8	68.3	65.4	67.8	67.6	<0.0001	5.47	<0.0001	0.905
	FCR	1.31	1.27	1.30	1.28	1.28	0.024	0.005	0.022	0.908
22-42 d	FI (g/b/d)	164	174	169	177	173	<0.0001	18.7	<0.0001	0.116
	BWG (g/b/dia)	107	114	113	115	115	0.002	16.4	0.002	0.885
	FCR	1.54	1.52	1.50	1.54	1.51	0.354	0.007	0.116	0.309
11-42 d	FI (g/b/d)	137	146	142	148	145	<0.0001	22.5	<0.0001	0.156
	BWG (g/b/dia)	91.9	99.1	97.3	99.8	99.5	<0.0001	19.6	<0.0001	0.872
	FCR	1.49	1.47	1.46	1.48	1.46	0.297	0.006	0.083	0.315

¹P+C, pellet plus crumble. ² Interaction between feed processing, 1 – Mash; 2 – Pellet; 3 – Crumble. ³ Comparison between the form of supply of pellet and fine feed.

Table 6. Food preference of broiler chickens in relation to pellet feed or crumbles (pellet: crumble) (trial 2)

Treatment	Age			
	11-21d	22-28d	29-35d	36-42d
P+C ¹ (separate)	0.82:1	2.29:1	1.38:1	1.85:1
P+C (mix)	0.99:1	1.04:1	0.95:1	1.00:1
p-value	0.055	<0.0001	<0.0001	<0.0001
SEM	0.043	0.207	0.055	0.126

¹P+C, pellet plus crumble.

Table 7. Carcass yield (CY), breast, leg, wings and abdominal fat, and body composition of broiler chickens fed mash, pellet and crumble (trial 2)

	Carcass yield (%)	Breast meat yield (%)	Leg yield (%)	Wings yield (%)	Abdominal Fat (%)	Protein deposition (g/kg)	Fat deposition (g/kg)
Mash	76.1	39.1	27.9	10.1	0.988	170	92.9
Pellet	76.7	39.9	27.6	9.90	1.23	161	101
Crumble	75.6	39.7	27.8	10.1	0.904	166	99.4
P+C ¹ (separate)	77.0	39.9	27.5	9.66	1.18	167	89.6
P+C (Mix)	76.5	39.4	27.7	9.85	1.07	159	87.6
P-value	0.119	0.458	0.897	0.001	0.002	0.153	0.409
SEM	0.168	0.164	0.120	0.044	0.029	1.58	2.60
1*2*3 ²	0.117	0.297	0.759	0.139	0.001	0.203	0.565
4*5 ³	0.379	0.296	0.646	0.143	0.207	0.100	0.823

¹P+C, pellet plus crumble. ² Interaction between feed processing, 1 – Mash; 2 – Pellet; 3 – crumble. ³ Comparison between the form of supply of pellet and crumble feed.

CAPÍTULO 5 – Considerações Finais

O avanço da tecnologia da produção de rações assim como a utilização de aditivos, tem um papel importante para que a indústria avícola alcance seus objetivos. Entretanto, para isso, o conhecimento científico contribui de forma significativa para o avanço da produção avícola de forma sustentável e lucrativa.

Esta tese elucida o aproveitamento energético das dietas de frangos de corte em duas situações, com a suplementação de fitase e com o processo de peletização. Para trazer um exemplo prático, um exercício foi realizado com o intuito de apresentar o impacto no custo de produção por kg de ganho de peso (GP) com a utilização de fitase (Cap 2). Neste exemplo, foi considerado uma produção de 100 mil kg de frangos (Tabela 1) e multiplicado pelo custo de produção por kg de GP.

Tabela 2. Simulação da produção de 100 mil kg de frango de corte

	C1	C1+1000FTU	C2	C2+1500FTU
R\$/kg de GP	3,07	3,01	3,07	2,99
Custo ração (R\$/100t)	307.000,00	301.000,00	307.000,00	299.000,00
Economia (R\$/100t)		6.000		8.000
Dif. (1000vs1500)			2000	

A utilização de fitase reduziu o custo de produção por kg de GP em 1,99 e 2,67% para 1000 e 1500FTU de fitase, respectivamente, suplementada aos controles, ainda que, nas dietas controle não foi utilizado fontes inorgânicas de fósforo, como normalmente é adotada na produção avícola. Além da redução do custo de produção por kg de GP, outro ponto importante é a redução da excreção de fósforo para o ambiente, através da melhora da digestibilidade deste nutriente.

Outro ponto importante que esta tese traz como implicação é a valoração da energia quando comparado em dois sistemas, de energia metabolizável (EM) e de energia líquida (EL). Normalmente, ao se formular as dietas quando se utiliza fitase os nutricionistas consideram a valoração da matriz da enzima utilizada, e por mais que o foco da fitase está relacionada a liberação de fósforo, também é considerado o cálcio, proteína e energia. Entretanto a valoração da energia tem sido pouco significante quando se leva em consideração o sistema de EM. Com isso, essa tese

mostrou os efeitos da utilização da energia no sistema de EL, que é o que realmente a ave utilizará para deposição de tecidos. Na figura 1, pode-se observar que para cada 1000 FTU de fitase é disponibilizado 32 kcal EMA/kg de ração, entretanto quando se compara com o sistema de EL (figura 2) a fitase disponibiliza 62kcal EL/kg de ração. Sendo assim, o sistema de EL possibilita melhor entender a utilização da energia para utilização das aves.

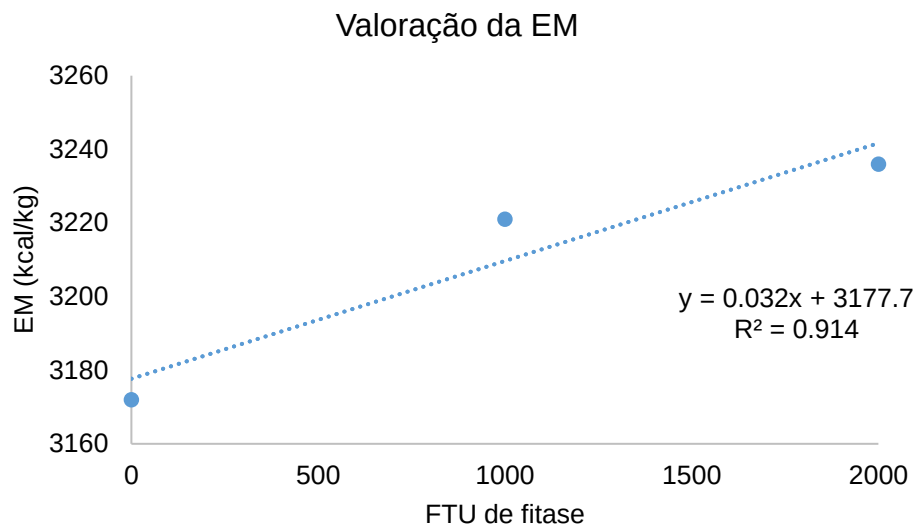


Figura 2. Valoração da EM com a suplementação de fitase

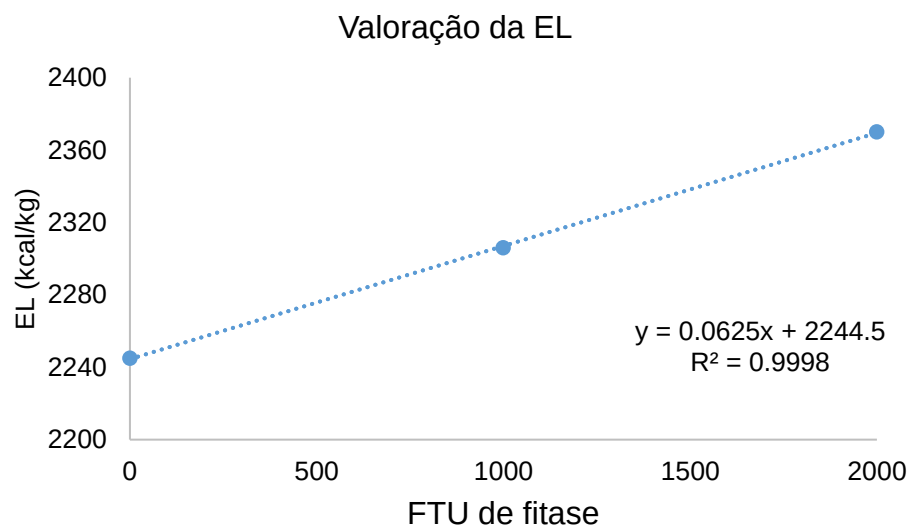


Figura 3. Valoração da EL com a suplementação de fitase

Além disso, no capítulo 4 foi demonstrado o efeito do processamento da ração, peletização, sobre a utilização da energia em frangos de corte. Fazendo um exercício, ilustrado na figura 3, pode-se destacar o efeito da forma física sobre o aumento da eficiência de utilização da energia, uma vez que a dieta na forma farelada apresentou maior incremento calórico em relação a dieta peletizada. Sendo assim, o sistema de EL permitiu melhor compreender o ganho da energia para as aves através do processamento e da forma física da ração.

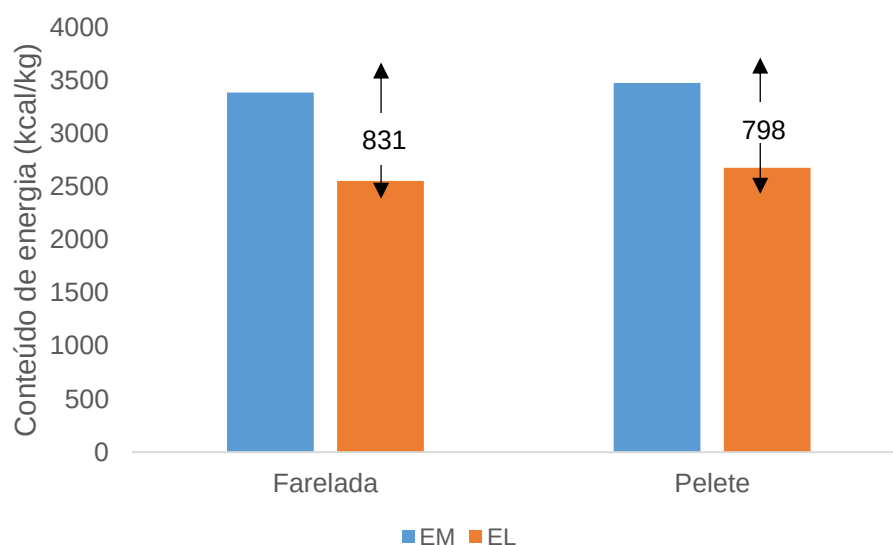


Figura 4. Valores de EM e EL de dietas farelada e peletizada