UNIVERSIDADE ESTADUAL PAULISTA - UNESP
CAMPUS DE JABOTICABAL

EFEITO DA PROTEÍNA BALANCEADA NA FORMAÇÃO DA FRANGA E NA PRODUÇÃO DE POEDEIRAS

Ingryd Palloma Teodósio da Nóbrega
Zootecnista

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Tese apresentada à Faculdade de Ciências Agrárias e Veterinárias – Unesp, Câmpus de Jaboticabal, como parte das exigências para obtenção do título de doutor (a) em Zootecnia.

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DADOS CURRICULARES DO AUTOR

INGRYD PALLOMA TEODÓSIO DA NÓBREGA, nascida no dia 15 de maio de 1993 em Princesa Isabel, Paraíba, Brasil, filha de Afonso da Nóbrega e Jailma Teodósio da Silva. Ingressou no curso de Zootecnia em 2011 na Universidade Federal Rural de Pernambuco, Campus de Serra Talhada, graduando-se em 2016, sob a orientação da Profª. Drª Thaysa Rodrigues Torres. Em agosto de 2016 ingressou no Programa de Pós-Graduação em Zootecnia da Universidade Estadual Paulista "Júlio de Mesquita Filho" – Faculdade de Ciências Agrárias e Veterinárias, Campus de Jaboticabal, sob a orientação do Prof. Dr. Edney Pereira da Silva, sendo bolsista CNPq, obtendo o título de mestre em Zootecnia em fevereiro de 2018. Em março de 2018, iniciou as atividades de doutorado em Zootecnia no mesmo programa sob orientação da Profa. Dra. Nilva Kazue Sakomura, sendo bolsista CAPES, submetendo-se à defesa da tese em abril de 2022.

Lembre-se da minha ordem: “Seja forte e corajoso! Não fique desanimado, nem tenha medo, porque eu, o Senhor, seu Deus, estarei com você em qualquer lugar para onde você for!”

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Dedico...

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Sumário

CAPÍTULO 1 – Considerações gerais 14
INTRODUÇÃO 14
REVISÃO DE LITERATURA 15
Poedeiras no ciclo longo de produção 15
Proteína e aminoácidos para aves de postura.. 16
Proteína dietética sobre a composição corporal de poedeiras 17
Proteína dietética sobre o desempenho produtivo..................................... 19
Efeito da proteína dietética sobre a qualidade dos ovos 20
Maturidade sexual 21
REFERÊNCIAS 22
CAPÍTULO 2 – Dinâmica do crescimento e características dos ovos em três cenários de proteína balanceada na dieta para galinha poedeira 28
Abstract.. 30
Introduction 32
Materials and Methods... 33
Results... 37
Discussion.. 40
Conclusion 46
CAPÍTULO 3 – Resposta de poedeiras à repleção e depleção de proteína balanceada na dieta 67
Abstract 69
Introduction 71
Materials and Methods 72
Results... 76
Discussion.. 79
Conclusion 85
CAPÍTULO 4 – Implicações.. 108

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CERTIFICADO

Certificamos que o projeto de pesquisa intitulado **"Diferentes relações de energia/proteína balanceada: Efeito da composição corporal no ciclo produtivo"**, protocolo nº 012598/2018, sob a responsabilidade da Prof.ª Dr.ª 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 extraordinária de 14 de fevereiro de 2019.

Vigência do Projeto	11/03/2019 a 15/04/2021
Espécie / Linhagem	Aves Lohmman LSL-LITE
Nº de animais	1000
Peso / Idade	± 1.800 kg / 101 semanas
Sexo	Fêmeas
Origem	Incubatório Lohmman do Brasil – Nova Granada/SP

Jaboticabal, 14 de fevereiro de 2019.

Fabiana Pilarski

Prof.ª Dr.ª Fabiana Pilarski
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EFEITO DA PROTEÍNA BALANCEADA NA FORMAÇÃO DA FRANGA E NA PRODUÇÃO DE POEDEIRAS

Resumo - Esta pesquisa foi realizada para estudar como níveis de proteína balanceada em cinco cenários de nutrição diferentes: controle, baixo, alto, depleção e repleção afetam o desenvolvimento, composição corporal, desempenho produtivo e qualidade dos ovos em galinhas poedeiras. Um total de 600 frangas da linhagem Lohmann-LSL Lite NA de 8 semanas foram distribuídas aleatoriamente em 5 tratamentos: Controle (**CC**, 100% da BP desde a recria até o período de postura), Baixo (**LL**, -20% BP Controle), Alto (**HH**, +20% BP Controle), repleção (**LH**, -20% de controle de BP na recria e +20% de controle de BP na postura) e depleção (**HL**, +20% de controle de BP na recria e -20% de controle de BP na postura). As rações experimentais para os períodos de recria (8-18 semanas) e postura (19 – 102 semanas) foram formuladas para atender as recomendações nutricionais do programa nutricional da Lohmann. As variáveis resposta avaliadas foram desempenho (produção de ovos, %; peso do ovo, g/ovo; massa de ovo, g; conversão alimentar, CA, g/g), composição corporal (porcentagem de proteína, gordura, cinzas e água) e qualidade do ovo (proporções de gema, albúmen, casca de ovo, resistência e espessura da casca). Análises de regressão foram realizadas para descrever as variações na composição corporal e dos ovos para os tratamentos **CC**, **LL** e **HH** ao longo da vida. Para avaliar a mudança nos níveis de BP no período completo de postura, foram realizados os seguintes contrastes ortogonais para desempenho, composição corporal e qualidade dos ovos: repleção (**LL** vs **LH**) e depleção (**HH** vs **HL**). Galinhas alimentadas com **LL** afetaram negativamente suas variáveis de desempenho sem afetar a composição corporal e a qualidade dos ovos; no entanto, as galinhas alimentadas com **HH** aumentaram o peso corporal (g/ave), gordura (%), massa de ovos (g/ave), enquanto a proteína corporal (%), cinzas (%) e água (%) foi reduzida. Galinhas poedeiras submetidas à repleção da BP melhoram o desempenho, aumentam o peso corporal (g/ave) e gordura (%) reduzindo proteína corporal (%) e cinzas (%); entretanto, a depleção da **BP** prejudicou o peso do ovo (g), a produção de ovos (g/ave) e a CA (g/g) sem afetar a composição corporal, a produção de ovos (%) e a qualidade dos ovos. Pode-se concluir que o regime prévio e contínuo do nível de **BP** no período de postura afeta o desempenho, a composição corporal e a qualidade dos ovos.

Palavras-chave: Conteúdo de composição corporal, componentes do ovo, produção de ovos, proteína balanceada, galinhas poedeiras

EFFECT OF BALANCED PROTEIN ON DEVELOPMENT AND EGG PRODUCTION IN LAYING HENS

Abstract

This research was carried out to study how a balanced protein levels in five different scenarios of nutrition: control, low, high, depletion, and repletion affects the development, body composition, laying rate, and egg quality in laying hens. A total of 600 8-week-pullets (Lohmann-LSL Lite NA) were randomized allocated in 5 treatments: Control (**CC**, 100% of BP from rearing to laying period), Low (**LL**, -20% BP Control), High (**HH**, +20% BP Control), repletion (**LH**, -20% BP control on rearing period and +20% BP control on laying period), and depletion (**HL**, +20% BP control on rearing period and -20% BP control on laying period). The experimental feeds for rearing (8-18 weeks) and laying (19 – 102 weeks) periods were formulated to meet the nutritional recommendations of nutritional program of Lohmann. The response variables evaluated were performance (Egg production, %; Egg weight, g/egg; egg output, g; fed conversion ratio, FCR, g/g), body composition (percentages of protein, fat, and ash), and egg quality (proportions of yolk, albumen, eggshell, shell strength and thickness). Regression analyses were performed to describe the variations in body and egg composition for treatment **CC**, **LL**, and **HH** along lifetime. To assess the change in **BP** levels over laying periods, the following orthogonal contrast for performance, body composition and egg quality were performed: repletion (**LL** vs **LH**) and depletion (**HH** vs **HL**). Hens fed with LL negatively affect their performance variables without affect the body composition and egg quality; however, hens fed with HH increase body weight (g/bird), fat (%), egg mass (g/bird) meanwhile body protein (%), ash (%), and water (%) was reduced. Laying hens subjected to **BP** repletion improve performance, increase body weight (g/bird) and fat (%) reducing body protein (%) and ash (%); however, **BP** depletion impair egg weight (g), egg output (g/bird), **FCR** without affecting body composition, egg production (%), and egg quality. It can be concluded that previous and continuous regime of **BP** level on laying period affect the performance, body composition and egg quality.

Keywords: Body composition content, egg components, egg production, balanced protein, laying hens

CAPÍTULO 1 – Considerações gerais

INTRODUÇÃO

Os geneticistas têm sido bem-sucedidos no aumento do número de ovos produzidos por ave alojada como evidenciado pelos relatórios produtivos nos últimos anos (Lohmann-LSL Management Guide 2011, Lohmann-LSL Lite Management Guide 2019). A extensão do ciclo de postura de 90 para 100 semanas de idade na última década resultou em um aumento de aproximadamente 100 ovos a mais por ave alojada, além do mais, as aves se tornaram mais eficientes, resultando em uma redução de mais de 10% na ração consumida por ovo produzido (Leentfaar, 2020).

O progresso genético e os ciclos mais longos de postura têm consequências diretas para a nutrição das aves, a qual exerce papel estratégico na busca por melhores índices produtivos, econômicos e ambientais. Nesse sentido, o aporte proteico no período de crescimento e produção é de extrema importância para a síntese de proteína no corpo, desenvolvimento dos órgãos reprodutivos e características de produção (Soares et. al, 2019; Bregendahl et al. 2008).

A restrição dos aminoácidos essenciais na fase que antecede a postura (pré-postura) pode provocar um atraso no início da postura e no peso inicial dos ovos, podendo inviabilizar a produção de ovos além das 90 semanas de idade (Keshavarz e Jackson,1992). Adicionalmente, a falta de equilíbrio dos aminoácidos essenciais na dieta pode afetar a performance das aves na fase de postura, o tamanho do ovo e a relação dos componentes interno (gema e albúmen) e externo (casca) do ovo, sendo estes fatores importantes para o produtor e para a indústria de ovos processados (Mousavi et al., 2013; Novak et al., 2004).

Trabalhos recentes demostraram que o aumento dos níveis de proteína balanceada na dieta de poedeiras impactou positivamente a produção de ovo, o peso do ovo, a massa de ovo e a relação dos componentes do ovo (Bregendahl et al., 2008; Bonekamp et al.,2010; Kumar et al., 2018 a, 2018 b). Kumar e colaboradores observaram que tanto o aumento como a redução da BP na dieta modificaram o teor de gordura abdominal e musculo peitoral de poedeiras no período de 27 a 66 semanas de idade.

Algumas pesquisas demonstraram que matrizes de frango de corte podem usar os componentes corporais para manter a produção de ovos quando os níveis nutricionais são inferiores a exigência das aves (Nonis e Gous, 2012; Nonis e Gous 2016; Vignale et al., 2016; Caldas et al. 2018). No entanto, não há evidências de como esse mecanismo ocorre em poedeiras leves e como a redução ou aumento da proteína balanceada na dieta influencia a dinâmica dos componentes do corpo e do ovo ao longo do tempo. Tendo em vista que o teor de proteína balanceada pode afetar o crescimento de matriz em crescimento, impactando o desempenho reprodutivo (Van Emous et al., 2012; Oviedo-Rondon et al., 2022), levantamos a hipótese de que baixos níveis de proteína balanceados afeta a formação de frangas e a composição corporal subsequente, levando a uma mudança no ciclo de postura a longo prazo e na relação dos componentes do ovo. Assim, os objetivos deste estudo foram:1-Compreender como os níveis de proteína induzem variações na composição corporal e nos componentes do ovo ao longo da idade das aves; 2-Avaliar o impacto da repleção e depleção da proteína balanceada na dieta de postura sobre a composição corporal, o desempenho e a qualidade dos ovos em poedeiras submetidas a dietas de alta e baixa nutrição proteica durante o período de recria.

REVISÃO DE LITERATURA

Poedeiras no ciclo longo de produção

Visto que os avanços genéticos estão orientados principalmente para o aumento da persistência de produção de ovos, o conceito de poedeiras de “vida longa” têm sido amplamente aplicados nos últimos anos. Em 2011, aves brancas produziam entre 350 e 360 ovos por ave alojada às 80 semanas de idade, podendo estender o ciclo de postura até as 90 semanas de idade, com a produção estimada entre 400 e 410 ovo por ave alojada (Lohmann-LSL Lite Management Guide 2011). Em apenas 8 anos, a produção de ovos aumentou para 371 e 377 ovos por ave alojada às 80 semanas de idade, e a extensão do ciclo de postura até às 95 semanas de idade, preconiza uma produção de 444 e 451 ovos por ave alojada (Lohmann-LSL Lite Management Guide 2019).

Atualmente a produção de ovos pode ser estendida até às 100 semanas de idade, com uma produção estimada de 500 ovos por ave alojada. (Leentfaar, 2020). O aumento de aproximadamente 100 ovos por ave alojada ao final da fase produtiva nos últimos 10 anos só é possível de ser alcançado quando a nutrição, o manejo, a saúde e o bem-estar animal são adequados.

A extensão do ciclo de postura tem consequências diretas para a nutrição, pois, as exigências nutricionais mudam em função do estado fisiológico das aves e a nutrição balanceada é indispensável para a expressão do potencial genético e para manutenção da qualidade dos ovos. Na fase de crescimento e desenvolvimento, o aporte de nutrientes da dieta é fundamental para que as frangas atinjam o peso alvo recomendado por volta das 14-16 semanas de idade e apresente composição corporal ideal para sustentar a produção de ovos além das 90 semanas (Bain et al., 2016). Neste sentido, uma curva de crescimento específica deve ser seguida principalmente em poedeiras no ciclo de produção longo, pois, qualquer desvio do peso alvo das frangas influenciará o peso médio do ovo durante a fase inicial de postura (Leeson e Summers, 1987) e a produção total de ovos ao final do período de produção (Bouvarel et al., 2011). Adicionalmente, o desenvolvimento da estrutura óssea das frangas é um fator crucial para manter a qualidade dos ovos no ciclo prolongado de postura e este tem sido um dos principais problemas enfrentados pelos produtores (Bain et al., 2016).

Proteína e aminoácidos para aves de postura

Os aminoácidos são utilizados pelas aves para o crescimento (tecidos estruturais e músculo), manutenção (tecidos e penas) e reprodução (produção de ovos). O fornecimento de aminoácidos essenciais (AAE) na dieta é calculado com base no conceito de proteína ideal descrito por Parsons e Baker (1994), o qual consiste em eleger um aminoácido como referência e basear as exigências dos outros aminoácidos em proporção desse aminoácido referência. A lisina é normalmente usada como aminoácido referência no conceito de proteína ideal por ser um aminoácido voltado principalmente para a deposição de proteína corporal, além de existir uma grande quantidade de publicações referentes às exigências de lisina para aves (Soares et al., 2019).

As recomendações de proteína ideal para poedeiras estão disponíveis na literatura e são utilizadas para formulação de dietas práticas e econômicas (Leeson e Summers, 2005; Bregendahl et al., 2008; Lemme, 2009 e Rostagno et al., 2017). Tendo em vista que a proteína balanceada visa manter as relações constantes entre os aminoácidos essenciais em relação a lisina, poucas pesquisas foram realizadas para avaliar o efeito da proteína balanceada sobre o desenvolvimento da franga, a composição corporal, o desempenho produtivo e a qualidade de ovos em poedeiras. Por outro lado, vários trabalhos com frango de corte têm demonstrado que a variação da proteína balanceada na dieta pode afetar o ganho de peso diário (Aftab et al., 2009), a conversão alimentar (Wijtten et al., 2004; Lemme et al., 2003) e os índices de deposição de proteína e gordura na carcaça (Corzo et al., 2002; Mlaba et al., 2015). Adicionalmente, os níveis de proteína balanceada na dieta afetam os níveis séricos de nitrogênio e consequentemente, a produção de ácido úrico, resultando em uma alteração na excreção de nitrogênio (Kumar et al., 2018b).

Proteína dietética sobre a composição corporal de poedeiras

O fornecimento ideal de proteína e aminoácidos dietético é um dos principais fatores para otimizar o crescimento e o desempenho produtivo das aves. Na fase de crescimento, a demanda proteica é orientada principalmente para a síntese de proteína no corpo, deposição do tecido magro e desenvolvimento dos órgãos reprodutivos (Alagawany et al., 2011; Alagawany, 2012; Soares et. al, 2019).

Na literatura geralmente as publicações avaliaram o efeito da proteína bruta sobre o peso corporal de frangas ao final da recria e o impacto disso no desempenho produtivo subsequente (Babiker et al., 2010; Oluwabiyi et al., 2022), contudo, nenhum conhecimento está disponível na literatura descrevendo como uma galinha moderna pode lidar com uma dieta deficiente durante o crescimento e seu impacto no ciclo de postura de longo prazo. Os trabalhos realizados com frangos de corte e matrizes em crescimento demonstram que tanto o peso como a composição corporal foram modificados em função dos níveis de proteína balanceada da dieta (Azevedo et al., 2021; Van Emous et al., 2015). Azevedo et al. (2021) observaram que o peso corporal de frangos de corte no período de 1 a 56 dias de idade apresentaram comportamento quadrático ao aumento dos níveis de BP na dieta, enquanto o teor de lipídios corporais aumentou linearmente na fase inicial e na fase de crescimento de forma quadrática, à medida que o teor de proteína da dieta foi reduzido. Van Emous et al. (2015) observou que matrizes alimentadas com baixa e alta proteína balanceada no período de 2 a 22 semanas de idade, apresentaram peso corporal semelhantes ao final da criação, entretanto a gordura corporal das aves alimentadas com baixa proteína aumentou e o músculo peitoral diminuiu em comparação as aves alimentadas com alta proteína, impactando a eclodibilidade durante a primeira fase de reprodução.

Em poedeiras no período de 27 a 66 semanas de idade, Kumar et al. (2018 b) avaliaram o efeito da proteína balanceada sobre a composição do corporal e demostraram que o peso corporal, o percentual de músculo peitoral e a gordura abdominal aumentaram com o aumento da ingestão de Lisina digestível com os demais aminoácidos balanceado. De acordo com Ekmay et al. (2014), a maioria da lisina absorvida da dieta é incorporada ao músculo esquelético, e pode ser fonte de AA para a formação de ovos. Caldas et al. (2016) verificaram maior taxa de degradação da proteína peitoral quando as aves estavam no pico de produção, sugerindo que as matrizes podem utilizar a proteína peitoral para sustentar a produção. Nonis e Gous (2012), observaram que as matrizes podem usar as reservas lipídicas corporais para manter a produção de ovos por curtos períodos quando o consumo de energia é inferior as necessidades.

Do mesmo modo, Caldas et al. (2018) ao avaliar a dinâmica dos componentes do corpo em matrizes alimentadas com uma dieta balanceada, constataram que o percentual de massa gorda no corpo reduziu no terço final da produção de ovos, além disso, o conteúdo mineral ósseo foi inferior no pico de produção (30 semanas) em comparação com 50 semanas de idade, sugerindo maior utilização dos minerais para a formação da casca do ovo nesse período. Compreender como as aves utilizam as reservas de minerais óssea é de suma importância em ciclos mais longos de postura, visto que, a osteoporose continua sendo um dos maiores desafios para manter o bem-estar das aves (Sandilands, 2011).

Apesar da dinâmica dos componentes do corpo de matriz serem conhecidos, estudos com poedeiras são escassos, principalmente quando considera o padrão moderno de produção de ovos além das 90 semanas de idade. Como a formação da franga em termos de composição corporal impacta significativamente o desempenho produtivo subsequente (Babiker et al., 2010; Van Emous et al. 2015), é importante compreender qual a influência disso em um longo ciclo de postura. Contudo, não há trabalhos que descrevam esse efeito. As pesquisas desenvolvidas com frango de corte e matriz observaram resultados semelhantes em que a gordura no corpo aumentou com a idade (Caldas et al., 2019; Van Emous et al., 2015), assim como Van Emous e colaboradores encontraram que a gordura abdominal foi maior ao término do período de produção.

Proteína dietética sobre o desempenho produtivo

O sucesso na fase de postura para poedeiras comerciais, tais como: boa persistência de postura, alta produção de ovos e bons índices de conversão, dependem de fatores que antecedem a fase de postura. A restrição da proteína dietética nessa fase pode acarretar atraso no início da postura e produção de ovos com menor peso (Keshavarz e Jackson,1992). E no período de postura, a deficiência ou excesso no fornecimento de proteína além de influenciar a degradação da proteína muscular ou aumentar a excreção de nitrogênio, podem afetar o consumo de ração e reduzir a produção de ovos (Costa et al., 2004).

O peso do ovo e a massa do ovo são parâmetros importantes do desempenho de galinhas poedeiras e são influenciados pelo consumo de aminoácidos das galinhas (Samie e Pur, 2007). Assim como o consumo de ração e a eficiência alimentar (Shim et al., 2013). Estudos desenvolvidos na década de 90 avaliaram o efeito da proteína bruta sobre o desempenho produtivo das aves (Pesti, 1991; Leeson e Caston, 1996), enquanto grande parte dos estudos atuais avaliaram o efeito dos níveis de aminoácidos digestíveis sobre as variáveis de interesse zootécnico (Novak et al, 2006; Wu et al., 2007; Lemme, 2009), contudo, há uma inconsistência nos resultados em função dos níveis de proteína dietética, atendendo ou não as exigências de aminoácidos digestíveis.

Kesharvarz e Austic, (2004) constataram que a redução de proteína bruta da dieta de 16% para 13%, sem a suplementação de aminoácidos, para poedeiras leves no período de 36 a 48 semanas de idade reduziu significativamente a produção de ovos, o peso dos ovos e a massa de ovos, afetando também o consumo de ração e consequentemente, piorando a conversão alimentar. Contudo, os autores observaram que a suplementação de metionina, lisina, triptofano, valina e isoleucina em dietas contendo 13% de proteína bruta, melhorou significativamente o desempenho produtivo e a conversão alimentar, porém, os níveis de aminoácidos essenciais em relação a lisina foram constantes entre os tratamentos.

O balanço ideal de aminoácidos em referência à lisina digestível para poedeiras está disponível na literatura na literatura (Leeson e Summers, 2005; Bregendahl et al., 2008; Lemme, 2009; Rostagno, 2017). Bonekamp et al. (2010) avaliaram diferentes níveis de proteína balanceada sobre o desempenho de poedeiras (Lohmann brown classic, Lohmann LSL classic) no período de 24 a 60 semanas de idade, neste estudo, os níveis de lisina digestível variaram entre 550 a 800 mg/ave/dia, enquanto a energia e minerais foram mantidos constantes. Os autores observaram com base na análise de regressão que a assíntota do peso do ovo, da produção diária de massa de ovos e da taxa de conversão alimentar não foram alcançadas com os maiores níveis de aminoácidos, o que implica que as poedeiras modernas requerem altos níveis de aminoácidos dietéticos para atingir o seu potencial genético. Assim como os níveis mais baixos de ingestão de proteína balanceada (550 e 600 mg de lisina/ave/dia) afetou a produção de ovos, o peso do ovo, a massa de ovo, o consumo de ração e a conversão alimentar.

Recentemente, Kumar et al. (2018 a) avaliaram o efeito dos níveis de proteína balanceados na dieta de poedeiras (Lohmann-LSL Lite) no período de 27 a 66 semanas de idade, variando a ingestão de lisina entre 550 e 850 mg/ave/dia. Os autores observaram que a produção de ovos, o peso dos ovos, a massa de ovo e o consumo de ração aumentaram, enquanto a conversão alimentar e a mortalidade diminuíram de forma quadrática com o aumento da ingestão de lisina digestível, além do mais, os autores ressaltaram que a exigência de proteína balanceada das aves variou conforme os critérios de resposta.

Efeito da proteína dietética sobre a qualidade dos ovos

Um ovo é constituído por aproximadamente 9,5% de casca, 22% de gema e aproximadamente 68,5% de albúmen do peso total do ovo (Bendezu et al., 2018). Tanto as qualidades externas (casca do ovo) quanto as internas (albúmen e gema) determinam a qualidade geral de um ovo. Com o avanço genético de aves de postura em ciclo de postura prolongado, a preocupação com o aumento do peso do ovo e a qualidade da casca é crescente entre nutricionistas e produtores de ovos comerciais, pois, a qualidade da casca do ovo diminui com o aumento do tamanho do ovo, corresponde ao aumento da idade das galinhas (Roland, 1979; Roberts e Ball, 2004).

Como o peso do ovo é influenciado principalmente pela quantidade e pelo balanço dos aminoácidos consumidos pelas galinhas, as medidas absolutas dos componentes do ovo também serão afetadas de maneira semelhante (Prochaska et al., 1996). Gunawardana et al. (2008), observaram que o peso da gema e do albúmen reduziram em função da redução da proteína bruta da dieta. Contudo, as proporções desses componentes não mudaram, mas o percentual da casca do ovo aumentou. Semelhantemente, Shim et al. (2013), relataram um aumento no peso absoluto da gema e uma redução do albúmen com o aumento do peso do ovo em função do aumento da proteína bruta em um programa de alimentação de quatro fases (21.6; 19.1; 16.3 e 16.1%) no período de 18 a 74 semanas de idade em comparação com as dietas reduzidas em 2 e 4% de proteína balanceada. Contudo, o percentual de gema em relação ao peso do ovo não foi afetado. Do mesmo modo, Mousavi et al. (2013) não observaram efeito dos níveis de proteína bruta sobre a percentagem dos componentes do ovo.

Em contrapartida, os níveis de ingestão de proteína bruta e aminoácidos podem influenciar o peso do ovo e afetar a qualidade da casca (Rocha et al., 2009; Jardim Filho et al., 2010). Além do mais, Kumar et al. (2018 b) observaram um declínio significativo no percentual da casca do ovo com os níveis crescente de lisina balanceada na dieta de 560 a 858 mg/ave/dia, atribuindo essa redução ao aumento do tamanho do ovo. Neste mesmo trabalho, os autores descrevem que o peso absoluto dos componentes e consequentemente o peso do ovo aumentaram com a ingestão de lisina digestível.

Maturidade sexual

O início da postura é definido pela idade média em que ocorre a produção do primeiro ovo (Age at firts egg, AFE). Já a maturidade sexual, é definida como a idade em que as aves atingem 50% da produção (Age at sexual maturity, ASM), sendo essa a medida mais usual para aves alojadas em grupos (Lewis et al., 2007). As frangas normalmente não iniciam seu ciclo de postura produzindo um ovo por dia, sendo assim, a AFE ocorre antes da ASM.

A taxa de maturação sexual é coordenada por hormônios como o hormônio luteinizante (LH) e o hormônio folículo estimulante (FSH), produzidos na glândula pituitária (Du et al., 2020). A liberação de LH e FSH é estimulada pelo hormônio liberador de gonadotrofina (GnRH), produzido no hipotálamo (Scanes, 1984). O primeiro também é chamado de fotorreceptor extra-retiniano ou encefálico profundo, pois a luz percebida nessa região do cérebro controlará a secreção de GnRH. Um sistema chamado eixo hipotálamo-hipofisário-gonadal permite que o GnRH alcance a glândula pituitária e inicie a liberação de LH e FSH (Du et al., 2020).

Outro hormônio que também controla a liberação de LH e FSH é o hormônio inibitório das gonadotrofinas (GnIH). O GnIH, também produzido no hipotálamo, é antagônico ao GnRH e impedirá a hipófise de liberar os hormônios LH e FSH (Ciccone et al., 2004). Tanto o GnRH quanto o GnIH são hormônios peptídicos, exigindo, portanto, um receptor no local de ação para realizar sua função. Os receptores de GnIH na hipófise diminuem em galinhas Lohmann entre 17 e 20 semanas de idade, enquanto os receptores de GnRH aumentam na mesma idade (Hanlon et al., 2021). Esses eventos podem aumentar a liberação de LH e FSH e contribuir para o início da postura.

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CAPÍTULO 2 – Dinâmica do crescimento e características dos ovos em três cenários de proteína balanceada na dieta para galinha poedeira

Este capítulo será apresentado de acordo com as normas da revista *Poultry Science*

Scientific section: Metabolism and Nutrition

Dynamics of growth and egg traits in three dietary balanced protein scenarios applied for laying hens

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Abstract

The objective of this study was to evaluate laying hens from eight to 102 weeks old, regarding the changes in performance, body, and egg components of laying hens produced in three scenarios of nutrition. A total of six-hundred Lohmann LSL-NA with 8 weeks old were allotted in 30 experimental units of 20 pullets each. Three treatments designed to contain crescent levels of balanced protein were randomly assigned to the experimental units, performing ten replicates per treatment. A control feed (C) was formulated to meet or exceed hen requirements. Then, two experimental feeds were formulated to contain 20% above (High, H) or below (Low, L) the dietary balanced protein (BP) used in the C feed. The experiment was conducted with hens from 8 to 102 weeks old. The response variables evaluated were cumulated feed intake (CFI, g) in the rearing and laying phases, hen-housed egg production (HHEP, %/hen-housed), daily feed intake (g/day), body weight (g), body composition (g of protein, fat, and ash), egg production (%), egg weight (g), egg mass (g), and egg components (percentages of yolk, albumen, and eggshell). The CFI was not affected by BP ($P>0.05$), whereas the HHEP reduced with the reduction in BP ($P<0.05$). Hens consuming the L feed reduced the egg production responses, affecting the egg mass ($P<0.05$). The hens from H group demonstrate an increase in the egg production responses when compared with L group. The body fat content of hens in the H group increased at the onset of lay and persisted until the end of this study, which suggests a necessity for further investigation. The changes in growth and lay performance, body and egg composition over the whole production cycle were demonstrated. The dietary balanced protein influences the body composition, egg production, egg weight, and egg mass of white laying hens. The increase of dietary balanced protein was related to an increase in body contents and egg weight, whereas hens consuming the low dietary balanced protein presented a lower body weight, leaner, and produced smaller eggs.

Keywords: pullet, amino acid, body component, egg component.

Introduction

Currently, there is a concept to keep laying hens for more extended periods in production, aiming to increase profitability and sustainability. Despite the benefits of doing so, controlling the excess body fat and eggshell quality in old laying hens is reported to be the main concern on poultry farms (Bain et al., 2016). The body fat and egg components are influenced by the feed offered (Nonis and Gous, 2016) and eggshell quality reduces as egg weight increases, which may be partially controlled with nutritional strategies (Pottguter, 2016; Bendezu et al., 2018). In addition, the feed offered to hens during the rearing phase may affect the development of reproductive organs (Silva et al., 2020), influencing the long-term laying cycle.

The ability of laying hens to overcome a nutritional deficiency or an imbalanced diet is not completely elucidated. Some effort was done to investigate the effects of a previous feed on the laying cycle phase (Leeson and Summers, 1981; Babiker et al., 2010) but little or no knowledge is available in the literature describing how a modern hen may deal with a deficient diet during the growth and its impact in a long-term laying cycle. This information is convenient for poultry nutritionists because they often change the feed formula to improve the economic return and sustainability of egg production farms. In this sense, protein is frequently investigated in poultry nutrition given its importance for growth (Leeson and Summers, 1989; Ocak and Sungu, 2009), egg production (Novak et al., 2006; Bregendahl et al., 2018), economic return (Novak et al., 2008; Lemme, 2009), and sustainability of the farm (Burley et al., 2013; Kumar et al., 2018 b). Because amino acids are the basic constituents of proteins and that essential amino acids should be offered in the feed in a proper ratio with lysine (Leeson and Summer, 2005), it seems reasonable to investigate the effects of balanced protein in a long-term egg production cycle.

Understanding the dynamics of body and egg components represents a step towards an effective way to improve long-term egg production since feeds should be formulated based on physiological needs and the response of laying hens. In a conventional poultry house, the feed offered is the only source of energy and nutrients for a laying hen. Therefore, a change in voluntary feed intake is the only mechanism that a hen can use to consume a proper amount of all nutrients. If they fail to do it so, body and egg components are expected to change (Silva et al., 2021; Novak et al., 2004) as the egg production (Castro et al., 2019). This highlights the importance to elucidate the dynamics of body and egg composition over different sets of nutrition scenarios.

The effects of dietary balanced protein for hens in the rearing phase and its cumulated influence in the long-term laying cycle were not investigated so far. In the context aforementioned, we hypothesized that balanced protein levels will affect the body and egg components, which leads to a shift in the long-term laying cycle; thus, the aim of the present research is to describe how laying hens respond to three levels of dietary balanced protein from eight to 102 weeks old.

Materials and Methods

Ethics Approval

All procedures described were approved by the Ethical Committee on the Use of Animals of the School of Agrarian and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil (Process 012598/2018; approved on 14 February 2019.

Bird husbandry

A total of 600 Lohmann LITE LSL-NA were obtained from a breeding company (Planalto Postura LTDA). The hatchlings were raised in conventional cages from one to seven weeks before being moved to wire rearing cages (375 cm^2 per pullet) from eight to 18 weeks. At 19 weeks, hens were transferred to wire-laying cages (563 cm^2 per hen). All cages were equipped with trough feeders and nipple drinkers. Hens received a corn-soybean meal-based diet to meet or exceed breeding company recommendations from one to seven weeks old. A feed program with three feeds was offered from eight to 18 weeks, for grower (eight to 11 weeks), developer (12 to 15 weeks), and pre-laying phases (16 to 18 weeks). At the laying phase, a feed program with five feeds was formulated according to the breeding company recommendations: layer one (19 to 26 wk-old), layer two (27 to 46 wk-old), layer three (47 to 66 wk-old), layer four (67 to 82 wk-old), and layer five (83 to 102 wk-old). Birds had free access to feed and fresh water throughout the trial. The lightning program adopted was 24L at the first week, reduced gradually to 12L:12D up to 10-wk-old, which was maintained until the pullets achieved 5% of egg production (20-wk-old). After the onset of egg production, the lightning program was gradually increased from 12 to 16 h of light by adding one hour per week and was then kept constant up to 102 weeks of age. In the period of zero to seven, eight to 18, and 19 to 102 w-old, the maximum temperatures recorded were 31, 26, and 27 °C, while the minimum temperature was 24, 17 and 19 °C, respectively. The maximum relative humidity of the air was 77, 79, and 84% while the minimum records were of 51, 57, and 50%, respectively for the same phases. One laying cycle was considered as 28 consecutive days.

Experimental design and feeds

Three treatments were randomly assigned to 30 experimental units of 20 pullets each, totaling ten replicates per treatment, performing a completed randomized design. Treatments consisted of three dietary levels of balanced protein (BP): 1- control feed (C-BP), formulated to meet or exceed breeding company recommendations; 2- reduction of

20% in dietary balanced protein (L-BP); 3- an increase of 20% in dietary balanced protein (H-BP). Dietary balanced protein was defined as a constant ratio of essential amino acids with lysine (Eits et al., 2005) and the ratio was the same proposed by the breeding company (Lohmann Tierzucht GmbH, Cuxhaven, Germany). Standardized ileal digestible lysine (SID-Lys) was used as a reference to produce the three levels of dietary balanced protein.

In the rearing phase, the control group consumed a feed containing 0.80, 0.70, and 0.74 % of SID-Lys for the grower, developer, and pre-layer phase, respectively (Table 1). Control feed in the laying phase contained 0.68, 0.66, 0.63, 0.61, and 0.58% of SID-Lys, respectively for each layer phase (Table 2). The remaining nutrients and energy in the feed were as recommended by the guideline (Lohmann-LSL Lite NA Management Guide 2019).

[Insert Table 1]

[Insert Table 2]

Performance data and egg components measurement

Number of eggs produced and mortality were daily recorded. Every week, all eggs produced were weighed and the egg mass was calculated. The feed leftovers were weighed fortnightly and adjusted by mortality to calculate the food intake. The cumulative feed intake was expressed on g/bird for the rearing (eight to 18 w-old) and the rearing plus laying phases (eight to 102 w-old). Hen-housed egg production was calculated based on number of eggs produced in the entire experiment period per number of housed hens at 19 w-old.

Body composition

On the first day of trial, eight birds per treatment were randomly selected and identified for body composition measurements by dual-energy X-ray absorptiometry (Hologic-QDR® model 13.4.2., Marlborough, USA). Throughout the experiment, the same hen was scanned on the last day of every feeding phase. Prior to each scan, hens were fasted for five hours, weighed, anesthetized with isoflurane (2%) diluted in 100% of oxygen, and positioned in dorsal decubitus with the wings and legs flexed (Alves et al., 2019). Measures collected were fat mass (g), lean mass (water + protein content, g), bone mineral content (g), and bone mineral density (g/cm^2). Data collected were converted to contents of body protein, fat, and ash by applying the equations published by Alves et al. (2019).

Egg components

At the end of each laying cycle (28 days), a total of nine eggs per experimental unit were collected (three eggs per three sequential days). In each day, the eggs were broken apart individually to measure the albumen, yolk, and shell weights. Before measurement, eggshell was washed with water and dried using a forced oven at 55°C for 24 hours. The percentages of albumen, yolk, and eggshell were then calculated.

Statistical analysis

Collected data was verified for outliers, normality, and homoscedasticity. The data of cumulative feed intake and hen-housed egg production was analyzed as One-Way ANOVA with a Tukey test to evaluate the differences between dietary balanced protein levels, using a generalized linear model. Two-factor repeated measure design was employed to determine the effects of dietary balanced protein over the time, using a mixed model. One factor is represented by the three groups receiving the different series of dietary balanced protein feeds and the other factor is the age of hens. Each experimental unit was the repeated measures factor. Differences were considered to be significant at a probability of 5%. The Statistical Analysis System (SAS Institute Inc., Cary, NC) was used to perform both a One-Way ANOVA and the Two-factor repeated measure analyses procedures. The data was analyzed considering 21 cycles of four weeks each.

To test whether the responses differed between dietary balanced protein levels over time, non-linear regression with groups was used, the groups being the dietary balanced protein (GENSTAT, VSN International 2017). The average data per replicate were treated as the experimental unit. Two exponential models were applied and that with the lower Akaike information criterion (AIC; Akaike, 1974) value was used to describe the response variable. The model used were:

Linear plus exponential: $y = A + B * (R^x) + C * x$

where A and C are the y-intercept and slope of the linear segment respectively, B is the y-intercept of the exponential segment, R is the exponential base, and x is the age in weeks.

Exponential: $y = A1 + B1 * (R1^x)$

where A1 + B1 is the y-intercept, R1 is the exponential base, and x is the age in weeks.

Results

The reduction and increase of dietary balanced protein in the laying feed did not affect the cumulative feed intake (p> 0.05, Table 3), being on average 4.44 kg (p = 0.986) and 67.7 kg of feed per bird (p = 0.485) in the growth and whole period, respectively. The reduction of dietary balanced protein affected the hen-housed egg production (p <0.01), with similar results between hens from C and H groups (P>0.05).

[Insert Table 3]

There was an interaction between dietary balanced protein and hens age for feed intake, egg production, and egg mass (P<0.05, Table 4). For feed intake, differences between treatments were observed only at 26 weeks of age (P<0.05). For egg production, differences were observed mainly at the beginning (first three laying cycles) and the end (after 74 w-old) of the laying cycle, whereas for egg mass the differences between groups of hens were consistent during the whole experimental period (Table 4).

[Insert Table 4]

The exponential equation was used to demonstrate the changes in feed intake and the line plus exponential equation egg production and egg mass with time (Table 5). The regression with groups identified that a single equation could be used to describe the feed intake between groups, whereas for egg production and egg mass the regression analysis indicate a necessity for different equations for laying hens inside each dietary balanced protein group (Figure 1).

[Insert Table 5]

[Insert Figure 1]

Differences in body weight influenced by dietary balanced protein were observed in 30 w-old hens (P<0.05, Table 6), with heavier hens in the H group, followed by the C and L groups, respectively. Laying hens in the higher dietary balanced protein feed was fatter (P<0.05) from 38 w-old and forward (Table 6). Body contents of ash and protein increased for all groups (P<0.05, Table 6). For body ash, differences were observed from

50 w-old and body protein from 30 w-old, and still relatively constant until the end of the trial.

[Insert Table 6]

The linear plus exponential function had the best fit for body weight and body contents, and it was used to investigate the differences between hens consuming the different feeds (Table 7). A common value for the coefficient R was sufficient to describe the changes in growth (P>0.05), despite the levels of dietary balanced protein, except for fat (P<0.05). However, the coefficients A, B, and C are different between groups (P<0.05) and specific values are necessary to properly describe the changes in body weight and body composition between hens consuming the different levels of dietary balanced protein (Figure 2).

[Insert Table 7]

[Figure 2 here]

Overall, egg weight of laying hens was affected by dietary balance protein content (P<0.05, Table 8). At 26 w-old, hens consuming the H feed produced heavier eggs, followed by hens from C and L groups, respectively. Notably, egg weight was similar between groups of hens from 30 to 42 w-old and from 54 to 66 w-old (P>0.05). The yolk percentage increased in all feed treatments as the hens aged, whereas the albumen and eggshell percentages reduced. Whatsoever, egg components were similar between groups of hens (P>0.05).

[Insert Table 8]

The exponential function had the best fit for egg weight and egg components (Table 9). The analysis indicates that all dietary balanced protein contents used in this study affected the egg weight ($P<0.05$) and all equation coefficients need to be changed to estimate the egg weight of hens according to dietary balanced protein. The range in dietary balanced protein levels applied in this study was not sufficient to change the concentration in egg components ($P<0.05$); therefore, a single exponential equation was used for each egg component. A tendency was observed for yolk percentage ($P=0.06$), suggesting an influence of dietary balanced protein in this egg component. The equation used to describe the albumen percentage had a low R^2 value, mainly because a drop in albumen percentage observed around 68 w-old was followed by a consecutive increase (Figure 3), which was poorly predicted with the exponential equation used.

[Insert Table 9]

[Figure 3 here]

Discussion

The aim of the study was to describe how three scenarios of protein levels elicited variations in the growth of laying hens and how such changes might affect long-term egg production and egg components. To our knowledge, this is the first study to investigate the influence of dietary balanced protein in laying hens, from the rearing period (eight w-old) until the end of laying cycle (102 w-old). Currently, there is a growing concept to keep laying hens for longer periods in production (Bain et al., 2016). Despite the benefits of doing so, maintaining the egg production and egg quality of a flock of older hens is a challenge. Pieces of evidence demonstrate that body weight of laying hens at the onset of lay may affect the entire egg production (Akbas e Takma, 2005) and the egg weight (Leeson and Summer, 1987; Lacin et al., 2008). Specifically, the body composition of laying hens at the beginning of laying phase could also affect the peak and persistence of egg production (Milisits et al., 2015). Dietary protein content is known to affect the growth of broilers and breeder pullets (Azevedo, et al., 2021; Van Emous et al., 2015), and considering the higher cost of dietary protein (Sakomura e Silva, 1998) and the trend in reducing the nitrogen excretion in poultry farms (Kumar et al., 2018b), it might be convenient to investigate the effects of dietary protein over the growing and laying phase. Assuming that essential amino acids are required in constant ratios with lysine, in this study the concept of balanced protein is used as proposed by Eits et al. (2003).

We observed that dietary balanced protein levels used in this study, poorly affected the daily feed intake of laying hens. A general theory for feed intake regulation was developed over the years (Pack, 1972, Emmans, 1981; Jhonston and Gous, 2006), suggesting that feed intake is regulated by the first limiting component in the feed, being energy or essential amino acids. Evidences demonstrate that feed intake of growing broiler chicken and pullet of broiler breeder is affected by dietary protein (Azevedo, et al., 2021; Van Emous et al., 2015). For laying birds, the feed intake regulation seems to be more complex because the consumed nutrients are also used for egg production. A model proposed by Fisher et al. (1973) and recently reviewed by Sakomura et al. (2015) accommodate this problem, splitting the amino acid requirement for maintenance and egg mass, which was called the Reading model. Those authors introduced a methodology to predict the requirements of essential amino acids (mg/hen/day), highlighting the importance to understand the mechanisms related to feed intake regulation. In the present study, the higher level of dietary balanced protein elicited an increase in egg mass. According to the cited model, egg mass will affect the essential amino acids requirement, which may explain why the feed intake did not reduce for laying hens in the H group. On the contrary, laying hens in the L group reduced the egg mass, therefore, a lower amino acid was needed for egg production, which may have impacted the feed intake.

On the other hand, when the cumulated feed intake is calculated per unit of egg produced, it was evidenced that laying hens in the L group consumed 130 g of feed per unit of egg produced, whereas C and H groups consumed 122 and 120 g of feed per egg produced. The feed intake per unit of body weight was 50.3, 49.7, and 44.2 kg of feed per kg of body weight for L, C, and H groups, respectively. Those results suggest that hens attempted to regulate their feed intake to compensate for the reduction in dietary balanced protein when the feed is deficient and reduce the feed intake when the dietary balanced protein is in excess. Recently, Kumar et al. (2008a) described a quadratic response of feed intake in function of the dietary balanced protein. Different from the study reported herein, laying hens (Lohmann-LSL) received a standard feed in the rearing phase. The laying hen current status, regarding body weight and body composition, seems to be an important factor that modulates their response and needs more attention in future studies.

Even though the reduction in dietary balanced protein may reduce the feed cost and nitrogen excretion (Burley et al., 2013; Fu et al., 2014), the feed intake per hen-housed egg increased. In this study, the number of eggs produced per hen-housed reduced 31 units for hens in the L group compared with C group. Therefore, feeding cost (feed price x feed intake), revenue, and viability of hens should be accounted to properly calculate the economic return. Viability observed in the L group was 85%, whereas for C and H groups were 90 and 93%, respectively. Laying hens consuming the L feed demonstrate an acute reduction in body fat after 54 w-old. The ovulation cycle was demonstrated to be dependent on plasma-free fatty acids and the body lipid seems to be the main blood source of fatty acids (Heald and Badman, 1963). We hypothesized that the ovulation cycle was affected by a reduction in body fat content, which reduces egg production in the L group. Eventually, a severe reduction of body fat might drastically affect the ovulation cycle and may stop egg production, reducing the viability of hens in the L group.

Laying hens from the H group had a body weight close to the recommendations in the guide-line (Lohmann Tierzucht GmbH, Cuxhaven, Germany), whereas the hens from the C and L groups were 200 g lighter. The variation in body weight was mostly due to body fat, since after sexual maturity there is a reduction in body protein deposition, and the change in body weight is given by variations in body fat deposition (Fisher and Gous, 2008; Nonis and Gous, 2016). The observations on body fat and egg production suggest that the hens in the H group did not have an excess of body lipid, since the laying performance was not affected. On the contrary, the persistence of egg production indicates that body fat in H group was favorable. Milisits et al. (2015) observed that laying hens with high body fat content at the onset of lay reduced the egg production in about 11 to 13 eggs when compared with hens with lower body fat content. There is a discussion about the importance of energy reserves as body fat; however, there is a lack of information on the desired body fat content that benefits longer-term egg production.

Using the first derivative of the linear plus exponential equation, the results demonstrate an increase in body fat content until 48 (L), 58(C) and 63(H) w-old, followed by a linear reduction until 102 w-old. As cited before, few differences were observed between groups, where laying hens from the H group seem to have a delay in body fat mobilization, regarding the age. After cited ages, the laying hens mobilize body fat, possible to maintain egg production, especially after 82 w-old, when the dietary metabolizable energy was reduced, as recommended by the guide-line. Nonis and Gous (2012), demonstrated that broiler breeders produce energy from body lipid if they are allowed to do it so, even though the concentration of dietary energy is above requirement, regulating their feed intake. Similarly, Caldas et al. (2018) observed a reduction of broiler breeders fat at the end of egg production phase. In line with our findings, Kumar et al. (2018b) observed a linear increase in body fat for laying hens consuming a feed with a crescent level of digestible lysine (raging from 560 to 858 mg/hen/day). However, the authors investigated the effect of balanced protein in Lohmann-LSL Lite NA until 66 w-old.

The body ash content of laying hens suggested that laying hens did not used mineral reserves to produce an egg, since the body ash increased until the end of laying cycle. The major portion of minerals used for egg formation is due to calcium carbonate necessary for eggshell formation since approximately 80% of eggshell is formed by this mineral (Liu et al., 2007). Around 99% of total body calcium is found in the bone ash (Rath et al., 2000). Evidences demonstrate that in a flock of older hens, there are individuals with a tendency to develop osteoporosis (Sandilands, 2011) and also an increase of eggs with thinner eggshells due to lower ability to uptake calcium and phosphorus from the intestinal lumen (Al-Batshan et al., 1994). In this study, dietary balanced protein seems to have a low or no effect over the dynamics of body ash contents. Apparently, the advanced ages of laying hens used in this study was not sufficient to elicit a negative consequence in the bone structure of laying hens; however, in all treatments the eggshell percentage reduced with age, probably due to the increase in egg size with the age of laying hens (Gunawardana et ai., 2008).

The dynamic of egg components observed over time was similar to reported by Bendezu et al. (2018) for white laying hens from 18 to 60 w-old. As the laying hens aged, the yolk percentage increased, and the albumen and eggshell reduced, which was consistent with reported literature (Johnston and Gous, 2007; Gous and Nonis, 2010). The dietary balanced protein had a tendency to influence yolk percentage. Compared with the L group, the hens from H group produced eggs with more yolk percentage. The contribution of dietary protein to yolk formation is probably related to phosvitin since this is the major protein molecule found in egg yolk. Around 56% of the amino acid found in the phosvitin is serine phosphorylated (Taborsky & Mok, 1967; Samaraweera et al., 2011). According to Huang and Dong (2019) it is believed that the role of phosvitin in the egg is related to embryo development, which reinforces the importance of such constituent in egg yolk. In the present study, the tendency of a lower percentage of yolk observed in eggs of the L group may be related to the lower amount of dietary serine, which is necessary to produce the phosvitin in the egg. With lower sources of dietary serine, essential amino acids might be used to overcome this deficiency.

Among the responses observed in this study, the body fat content between laying hens consuming the different levels of balanced protein was unexpected. Most reports in the literature demonstrate that growing birds would increase body lipid content when they are offered a low balanced protein feed (Azevedo et al., 2021; Van Emous et al., 2015). The opposite result is reported when a growing bird consumes a high balanced protein feed. The control of body fat content on laying birds seems to be more complex and the prediction of body fat in laying hens should be done with caution. The increase in egg yolk percentage may contribute to a higher value of body fat content since 34% of egg yolk is constituted of lipid (Tang et al., 2015). Hocking (2004), investigated the effect of body weight and feed intake over the ovarium follicular dynamics and found that feed-restricted broiler breeders reduced the number of yellow follicles. We did not find a similar study for laying hens, which would contribute to a better understanding of the results observed, but we hypothesize that a pullet raised with high level of dietary balanced protein feed may increase the number and the weight of yellow follicles in the ovarium, resulting in more body fat content in laying hens.

Conclusion

As expected, the dietary balanced protein influenced the dynamics of performance, body content, egg production, and egg mass of laying hens in the rearing and laying phases. The performance of laying hens increased with higher levels of balanced protein but other responses such as the feeding cost also influence the economic return and need to be considered to make a nutritional decision. The hen-housed egg production reduced in laying hens consuming a feed with low levels of dietary balanced protein. In this study, body ash was not mobilized, indicating that the minerals consumed was sufficient for egg production and that the dietary balanced protein did not influenced this variable. On the contrary, a mobilization of body fat was observed, being more evident at the end of laying cycle. The dietary balanced protein levels investigated in this study slightly affected the yolk percentage but had no influence on albumen and eggshell percentages. More persistence of egg production was observed for laying hens consuming a high dietary balanced protein feed.

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Declaration of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Software and data repository resources

None of the data was deposited in an official repository. The data can be obtained from the authors upon request.

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Table 1. Composition and nutritional content of experimental feeds in rearing period

	Grower (8-11 weeks)			Developer (12-15 weeks)			Pre-layer (16-18 weeks)		
Ingredients	Ideal	Low	High	Ideal	Low	High	Ideal	Low	High
Corn (8.8%)	62.2	68.0	56.3	58.2	63.0	53.4	55.5	61.0	49.9
Soybean meal (45%)	23.3	15.0	31.6	16.9	10.0	23.8	17.1	10.0	24.2
Wheat bran	10.1	13.0	7.10	17.5	20.0	15.0	17.0	20.0	14.0
Potassium carbonate	0.120	0.240	-	0.105	0.210	-	0.143	0.280	0.005
Corn gluten (60%)	-	-	-	1.50	1.50	1.50	1.00	-	2.00
Meat and Bone Meal 48%	-	-	-	2.66	2.66	2.66	3.42	2.97	3.87
Soy oil	0.825	0.150	1.50	0.935	0.370	1.50	1.05	0.685	1.42
Dicalcium phosphate	1.18	1.17	1.20	0.150	0.140	0.161	0.105	0.210	-
Limestome	1.39	1.46	1.32	1.32	1.38	1.26	3.87	3.98	3.75
Salt	0.353	0.287	0.420	0.260	0.215	0.306	0.236	0.215	0.256
Sodium Bicarbonate	0.100	0.200	-	0.133	0.200	0.065	0.154	0.198	0.110
Vit. and Min. supplement[1]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
DL-Methionine (99%)	0.108	0.055	0.161	0.057	-	0.114	0.078	0.045	0.111
L-Lysine HCl (78%)	0.062	0.100	0.024	0.019	0.038	-	0.061	0.095	0.027
L-Threonine (98.5%)	0.015	-	0.031	-	-	-	-	-	-
Choline chloride (60%)	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Total	100	100	100	100	100	100	100	100	100
---------- Calculated nutritional content (%) ----------									
Met. energy (kcal/kg)	2881	2883	2880	2860	2860	2860	2778	2778	2778
Crude protein (%)[2]	17.1	14.3	20.0	17.1	14.7	19.5	17.0	13.9	20.2
Dig. Lysine	0.803	0.645	0.960	0.700	0.560	0.840	0.742	0.593	0.890
Dig. Methionine + cysteine	0.592	0.476	0.709	0.544	0.434	0.653	0.552	0.442	0.662
Dig. Threonine	0.589	0.475	0.703	0.553	0.471	0.635	0.546	0.441	0.652
Dig. Tryptophan	0.187	0.148	0.226	0.171	0.139	0.203	0.169	0.133	0.206
Dig. Isoleucine	0.628	0.496	0.760	0.589	0.480	0.698	0.581	0.444	0.719
Dig. Valine	0.698	0.568	0.827	0.680	0.573	0.787	0.672	0.532	0.812
Calcium	1.04	1.04	1.04	1.05	1.05	1.05	2.08	2.08	2.08
Available Phosphurus	0.460	0.460	0.460	0.430	0.430	0.430	0.457	0.457	0.457
Sodium	0.180	0.180	0.180	0.170	0.170	0.170	0.170	0.170	0.170

[1]Content (per kg of product) Vit. A 4,850,00 Ul, Vit. D3 1,350,000 Ul, Vit. E 8,500 Ul, Vit. K3 1,395 mg, Vit. B1 1,000 mg, Vit. B2 2,570 mg, Pantothenic acid 5,295 mg, Vit. B6 1,525 mg, Vit. B12 7,500 mcg, Niacin 19.45 g, Folic acid 500 mg, Biotin 41.50 mg, Choline chloride 75 g, Iron 22 g, Copper 4,500 mg, Manganese 25 g, Zinc 25 g, iodine 500 mg, selenium 125 mg, Phytase 300,000 FYT.

[2]Values represent the mean analyzed composition by near-infrared spectroscopy (NIR).

Table 2. Composition and nutritional content of experimental feeds in the laying phase

	Layer 1 (19-26 weeks)			Layer 2 (27-46 weeks)			Layer 3 (47-66 weeks)			Layer 4 (67-82 weeks)			Layer 5 (83-102 weeks)		
Ingredients	Control	Low	High	Control	Low	High	Control	Low	High	Control	Low	High	Control	Low	High
Corn (8.8%)	59.0	65.0	53.0	61.1	67.0	55.3	61.9	67.5	56.2	63.3	68.5	58.0	63.4	68.7	58.1
Soybean meal (45%)	16.1	10.1	22.0	18.4	13.0	23.9	16.9	11.6	22.3	16.6	11.7	21.4	16.1	11.9	20.2
Wheat bran	3.75	6.00	1.49	2.35	4.70	-	2.50	5.00	-	2.50	5.00	-	2.50	5.00	-
Potassium carbonate	0.450	0.560	0.340	0.386	0.470	0.302	0.425	0.525	0.325	0.408	0.500	0.315	0.425	0.510	0.340
Corn gluten (60%)	7.50	5.00	10.0	5.43	2.55	8.30	5.76	2.95	8.56	5.01	1.95	8.07	4.54	1.10	7.98
Soy oil	1.07	0.890	1.25	0.725	0.610	0.840	0.680	0.570	0.790	0.566	0.580	0.552	0.670	0.660	0.680
Dicalcium phosphate	1.31	1.30	1.32	1.16	1.14	1.17	1.11	1.09	1.14	1.11	1.09	1.14	1.01	0.98	1.04
Limestome	9.11	9.17	9.06	9.46	9.51	9.41	9.77	9.82	9.72	9.77	9.82	9.72	10.4	10.4	10.3
Salt	0.307	0.279	0.336	0.323	0.290	0.356	0.292	0.275	0.310	0.270	0.260	0.280	0.275	0.280	0.270
Sodium Bicarbonate	0.155	0.200	0.110	0.132	0.183	0.080	0.139	0.168	0.110	0.174	0.190	0.157	0.166	0.160	0.172
Vit and Min supplement[1]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
DL-Methionine (99%)	0.066	0.041	0.090	0.065	0.047	0.082	0.052	0.036	0.068	0.048	0.038	0.059	0.044	0.038	0.050
L-Lysine HCl (78%)	0.099	0.119	0.078	0.015	0.029	-	0.019	0.038	-	0.011	0.022	-	-	-	-
Choline chloride (60%)	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Washed sand	0.822	1.023	0.621	0.075	0.150	-	0.165	0.159	0.171	0.026	0.052	-	0.248	-	0.495
Total	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
							---------- Calculated nutritional content (%) ----------								
Met. energy (kcal/kg)	2795	2795	2795	2785	2785	2785	2785	2785	2785	2785	2785	2785	2770	2770	2770
Crude protein[2]	18.1	15.6	21.4	17.0	14.6	20.6	16.1	13.3	20.2	16.0	11.7	18.7	14.8	11.4	18.7
Crude Fibre	2.24	2.22	2.25	2.36	2.50	2.22	2.19	2.22	2.16	2.19	2.23	2.14	2.16	2.23	2.09
Dig. Lysine	0.680	0.544	0.816	0.655	0.524	0.786	0.625	0.500	0.750	0.605	0.484	0.726	0.580	0.464	0.696
Dig. Methionine + cysteine	0.600	0.480	0.720	0.580	0.464	0.696	0.560	0.448	0.672	0.540	0.432	0.648	0.520	0.416	0.624
Dig. Threonine	0.571	0.459	0.683	0.570	0.459	0.681	0.555	0.446	0.664	0.540	0.433	0.647	0.524	0.420	0.627
Dig. Tryptophan	0.157	0.123	0.192	0.163	0.131	0.196	0.156	0.124	0.188	0.153	0.123	0.183	0.148	0.121	0.176
Dig. Isoleucine	0.633	0.486	0.781	0.630	0.484	0.776	0.612	0.468	0.755	0.590	0.450	0.731	0.570	0.434	0.707
Dig. Valine	0.716	0.563	0.869	0.706	0.553	0.859	0.689	0.539	0.839	0.666	0.518	0.813	0.644	0.499	0.789
Calcium	3.95	3.95	3.95	4.05	4.05	4.05	4.15	4.15	4.15	4.15	4.15	4.15	4.35	4.35	4.35
Available Phosphurus	0.440	0.440	0.440	0.410	0.410	0.410	0.400	0.400	0.400	0.400	0.400	0.400	0.380	0.380	0.380
Sodium	0.175	0.175	0.175	0.175	0.175	0.175	0.165	0.165	0.165	0.165	0.165	0.165	0.165	0.165	0.165

[1]Content (per kg of product) Vit. A 4,850,00 Ul, Vit. D3 1,350,000 Ul, Vit. E 7,785 Ul, Vit. K3 1,195 mg, Vit. B1 1,200 mg, Vit. B2 3,000 mg, Pantothenic acid 4,236 mg, Vit. B6 1,522 mg, Vit. B12 7,708 mcg, Niacin 16.21 g, Folic acid 500 mg, Biotin 41.50 mg, Choline chloride 93.75 g, Iron 22 g, Copper 4,500 mg, Manganese 25 g, Zinc 25 g, iodine 500 mg, selenium 125 mg, Phytase 300,000 FYT.

[2]Values represent the mean analyzed composition by near-infrared spectroscopy (NIR).

Table 3. Cumulative feed intake and hen-housed egg production (± standard deviation) of laying hens from 8 to 102 weeks old in response to three dietary balanced protein feeds.

Treatments[1]	Cumulative feed intake, kg/bird		Hen housed egg production[2], und
	8 to 18 weeks	8 to 102 weeks	
C	4.44 ± 170	67.2 ± 1.82	516 ± 15[a]
L	4.45 ± 131	67.4 ± 2.88	485 ± 24[b]
H	4.43 ± 151	68.4 ± 1.72	529 ± 22[a]
P-value	0.986	0.485	0.001

[1]C- control, formulated to meet the nutritional recommendation of Lohmann-LSL guideline; L- 20% reduction of BP from C; and H- 20% increase of BP from C.
[2]Distinct letter in the same column is significantly different by Tukey's test for each phase.

Table 4. Performance of laying hens from 8 to 102 weeks old in response to three dietary balanced protein feeds.

Age,	Feed intake, g/bird/day					Egg production, %					Egg Mass, %				
weeks	C[1]	L	H	SEM[2]	P-value	C	L	H	SEM	P-value	C	L	H	SEM	P-value
11	56.0	55.8	53.5	1.16	0.3930	-	-	-	-	-	-	-	-	-	-
15	56.4	56.9	56.8	1.39	0.9740	-	-	-	-	-	-	-	-	-	-
18	61.6	61.8	63.4	1.61	0.7450	-	-	-	-	-	-	-	-	-	-
22	79.2	75.9	80.2	1.26	0.1090	36.6	27.0	45.7	1.51	<.0001	17.4	13.1	20.9	0.927	<.0001
26	97.5	95.6	102	1.21	0.0060	94.3	91.9	96.8	1.41	0.0320	52.8	49.7	55.6	0.927	0.0010
30	104	102	103	0.94	0.7870	96.7	90.4	97.5	1.32	<.0001	56.8	51.4	58.4	0.927	<.0001
34	109	105	109	1.20	0.1570	97.3	92.6	97.4	1.32	0.0100	58.6	54.3	60	0.994	0.0010
38	109	110	110	1.25	0.7140	97.3	94.4	98.0	1.32	0.0990	60.6	56.7	61.9	0.927	0.0020
42	107	106	110	1.08	0.1850	97.7	94.6	98.0	1.32	0.1140	60.6	56.9	61.3	0.927	0.0060
46	108	109	108	0.78	0.6660	97.3	93.8	96.9	1.32	0.1130	60.4	56.1	61.4	0.927	0.0010
50	109	110	110	1.09	0.8910	96.5	91.9	95.8	1.41	0.0240	60.8	55.7	60.6	0.927	0.0010
54	109	110	111	0.86	0.4860	97.1	92.8	97.3	1.41	0.0220	61.6	57.2	61.6	0.927	0.0040
58	108	110	110	0.92	0.5500	95.8	91.7	94.9	1.51	0.0660	60.6	56.3	60.7	0.995	0.0040
62	111	113	113	1.07	0.6690	92.2	88.7	92.2	1.41	0.0790	59.5	56.1	60.1	0.994	0.0190
66	112	115	115	1.04	0.2590	95.8	95.1	94.9	1.32	0.8650	63.0	61.4	62.9	0.927	0.4860
70	113	115	115	0.900	0.4980	95.4	93.4	94.7	1.51	0.5670	61.9	58.7	62.0	0.995	0.0460
74	113	113	115	1.28	0.5940	95.1	92.1	94.8	1.51	0.2160	62.3	56.8	62.5	0.927	<.0001
78	109	109	111	1.04	0.5720	93.3	89.5	95.4	1.51	0.0070	61.1	55.0	62.5	0.927	<.0001
82	101	103	103	1.65	0.7180	91.8	86.4	90.9	1.41	0.0070	60.1	53.5	59.1	0.927	<.0001
86	107	106	110	1.29	0.1760	86.4	82.5	89.3	1.51	0.0020	56.1	49.5	59.1	0.994	<.0001
90	109	108	107	1.46	0.6820	87.4	79.6	92.6	1.61	<.0001	57.4	49.4	61.3	0.994	<.0001
94	107	109	107	1.73	0.6230	84.5	81.3	89.9	1.41	<.0001	55.4	49.9	60.2	0.994	<.0001
98	109	114	111	1.97	0.2150	83.2	82.7	87.7	1.61	0.0190	54.9	51.7	57.1	0.994	0.0020
102	109	111	110	1.90	0.7850	79.9	81.0	85.0	1.41	0.0160	52.7	50.7	56.1	0.995	0.0020
Main effects															
Age					<.0001					<.0001					<.0001
Balanced Protein					0.7210					<.0001					<.0001
Interaction					0.0070					<.0001					<.0001

[1]C- control, formulated to meet the nutritional recommendation of Lohmann-LSL guide-line; L- 20% reduction of BP from C; and H- 20% increase of BP from C.

[2]Standard error of the mean.

Table 5. Coefficients from an exponential equation for feed intake of laying hens from 8 to 102 weeks old and coefficients from a linear plus exponential equation for egg production and egg Mass of laying hens from 19 to 102 weeks old in response to three dietary balanced protein feeds.

Parameters	Feed intake, g/bird/day	Egg production, %			Egg Mass, g		
		C[1]	L	H	C	L	H
A	111.4	108.0	102.0	104.0	65.30	61.40	63.10
B	-163.1	-5150000	-51900000	-272000000	-63700	-77000	-489000
C	-	-0.2280	-0.1940	-0.1510	-0.0870	-0.0980	-0.0380
R	0.9210	0.5990	0.5410	0.4960	0.7200	0.7140	0.6530
SEM[2]	6.040		2.920			2.960	
R2[3]	88.80		95.00			91.00	

[1]C- control, formulated to meet the nutritional recommendation of Lohmann-LSL guide-line; L- 20% reduction of BP from C; and H- 20% increase of BP from C.
[2]Standard error of the mean.
[3]Coefficient of determination.

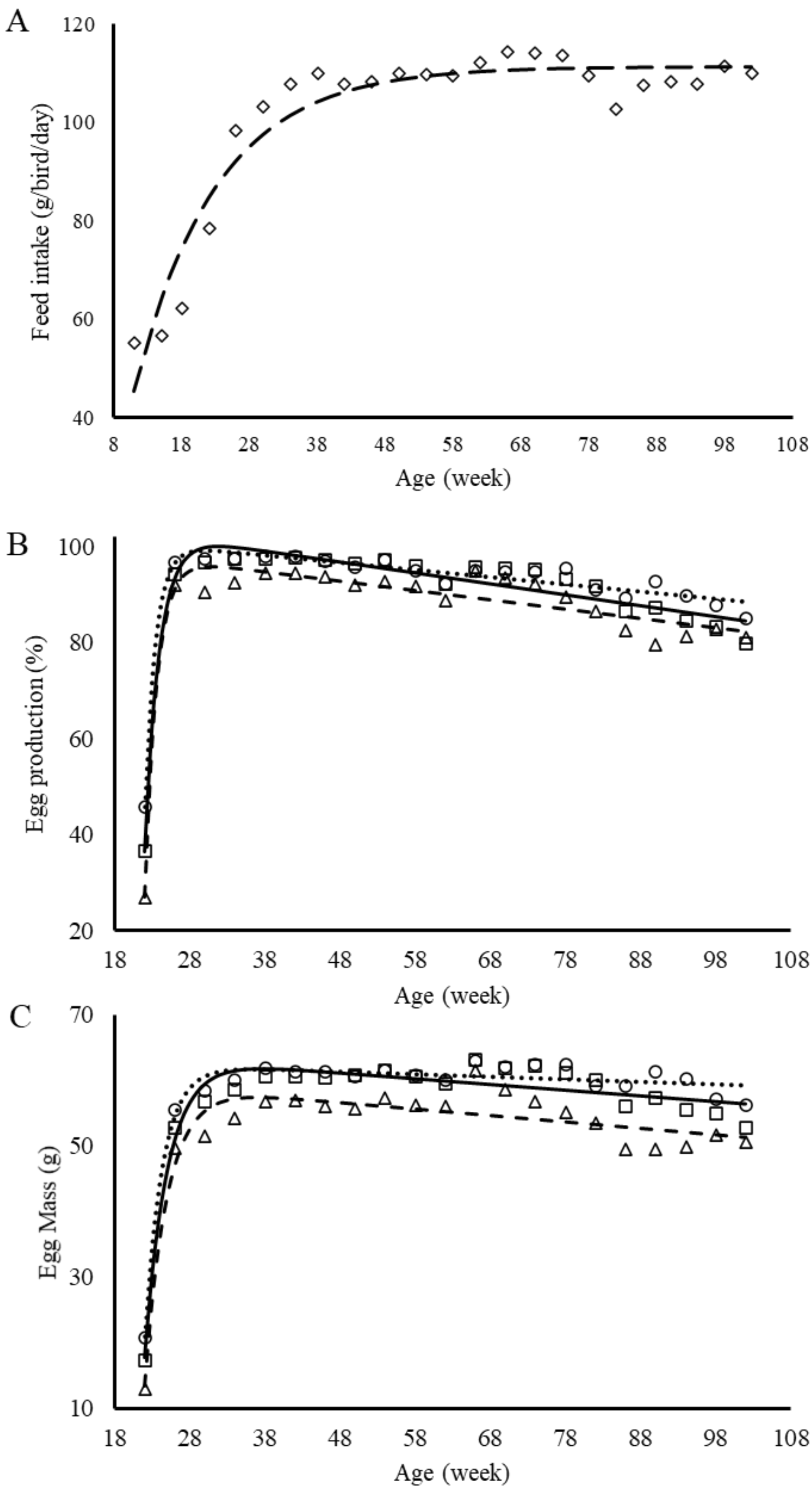

Figure 1**.** Observed and predicted feed intake (A: ◊, – –) of laying hens from 8 to 102 weeks old and of egg production (B) and egg mass (C) of laying hens from 19 to 102 weeks old in response to three dietary balanced protein feeds: control (□, —), low (Δ, - -), and high (○, ···).

Table 6. Body weight and body composition of laying hens from 8 to 102 weeks old in response to three dietary balanced protein feeds.

Age,	Body weight, g					Ash, g					Fat, g					Protein, g				
weeks	C[1]	L	H	SEM[2]	P-value	C	L	H	SEM	P-value	C	L	H	SEM	P-value	C	L	H	SEM	P-value
8	518	530	527	8.23	0.9820	18.3	18.9	18.0	0.401	0.8248	38.4	41.1	41.8	1.66	0.8587	73.0	73.2	74.0	1.52	0.9171
11	894	899	926	13.2	0.8608	29.6	28.6	30.3	0.594	0.6182	80.3	81.6	87.7	2.25	0.9586	132	131	140	1.93	0.6790
15	1,010	1,046	1,038	26.1	0.8312	33.9	33.4	34.3	3.89	0.8631	94.6	102	101	4.59	0.9544	153	154	159	3.87	0.8188
18	1,152	1,148	1,229	28.9	0.2692	39.3	37.1	40.2	0.600	0.1094	116	119	139	5.63	0.5461	178	173	189	5.08	0.4017
22	1,370	1,366	1,471	38.5	0.1935	45.4	45.7	47.2	12.1	0.5528	146	154	163	11.2	0.7326	218	214	227	7.77	0.5049
26	1,398	1,384	1,504	46.9	0.1139	45.2	45.7	47.2	9.23	0.4427	176	186	215	19.8	0.3234	214	206	223	6.50	0.2032
30	1,422	1,374	1,533	49.8	0.0349	47.1	45.4	47.7	11.9	0.3555	167	159	198	15.8	0.1676	220	212	237	7.07	0.0168
34	1,477	1,412	1,564	52.5	0.0514	46.3	45.4	47.7	12.8	0.3993	181	174	217	16.5	0.0970	226	215	235	6.38	0.0651
38	1,479	1,449	1,616	46.9	0.0186	46.1	46.7	49.4	5.00	0.1220	170	184	234	11.9	0.0151	231	223	245	6.61	0.0548
42	1,485	1,466	1,641	47.0	0.0092	47.1	47.1	49.7	9.32	0.1954	184	182	232	15.4	0.0351	234	226	252	6.34	0.0086
46	1,514	1,485	1,657	45.2	0.0141	48.7	47.4	50.6	4.63	0.1569	191	198	243	15.7	0.0402	241	223	250	6.29	0.0096
50	1,507	1,480	1,681	46.9	0.0026	46.5	47.7	51.3	12.9	0.0130	205	195	268	14.5	0.0025	224	224	254	6.72	0.0006
54	1,523	1,472	1,689	52.2	0.0011	47.2	47.3	51.3	11.9	0.0137	192	195	268	16.3	0.0014	227	220	248	6.01	0.0031
58	1,548	1,470	1,695	43.7	0.0014	47.8	47.2	51.3	4.97	0.0331	208	180	238	16.1	0.0312	243	227	262	5.57	0.0004
62	1,484	1,445	1,679	52.7	0.0012	46.8	47.3	50.6	13.5	0.0499	183	175	243	17.8	0.0119	236	232	259	7.02	0.0070
66	1,550	1,479	1,724	58.5	0.0004	48.7	47.7	52.4	13.8	0.0139	197	182	263	17.8	0.0006	242	229	259	7.74	0.0077
70	1,460	1,454	1,703	49.2	0.0001	47.7	49.2	50.2	12.7	0.5501	196	176	286	20.2	<.0001	235	225	242	7.14	0.2201
74	1,467	1,454	1,743	47.3	<.0001	46.8	49.6	52.4	12.8	0.0321	186	182	280	17.6	<.0001	229	220	256	6.67	0.0002
78	1,483	1,441	1,740	44.0	<.0001	47.9	50.0	52.3	14.4	0.0478	213	169	281	18.6	<.0001	223	223	253	6.17	0.0016
82	1,462	1,397	1,696	41.5	<.0001	48.3	47.8	52.7	4.86	0.0121	180	162	256	15.3	<.0001	226	221	253	6.23	0.0017
86	1,463	1,373	1,733	40.4	<.0001	50.7	48.9	53.6	10.9	0.0431	194	141	250	16.8	<.0001	233	213	261	5.68	<.0001
90	1,452	1,357	1,714	32.2	<.0001	48.1	51.9	54.5	15.4	0.0072	184	163	233	19.2	0.0553	241	228	262	9.90	0.0870
94	1,477	1,374	1,672	48.2	<.0001	49.5	50.5	53.9	13.8	0.0697	172	137	212	14.2	0.0061	235	216	260	6.76	<.0001
98	1,501	1,398	1,675	44.6	0.0002	51.1	50.5	53.2	10.2	0.3497	172	139	214	12.7	0.0079	230	222	260	5.21	0.0004
102	1,344	1,341	1,689	37.1	<.0001	48.3	48.7	54.7	5.81	0.0018	149	137	229	14.5	0.0051	219	213	266	7.01	<.0001
Main effects																				
Age					<.0001					<.0001					<.0001					<.0001
Balanced Protein					0.0032					0.0704					0.0147					0.0100
Interaction					<.0001					0.0072					<.0001					<.0001

[1]C- control, formulated to meet the nutritional recommendation and feeding program of Lohmann-LSL guide-line; L- 20% reduction of BP from C; and H- 20% increase of BP from C.
[2]Standard error of the mean.

Table 7. Coefficients from linear plus exponential equation for body weight and body composition of laying hens from 8 to 102 weeks old in response to three dietary balanced protein feeds.

Parameters	Body Weight, g			Ash, g			Fat, g			Protein, g		
	C[1]	L	H	C	L	H	C	L	H	C	L	H
A	1638	1631	1712	46.35	46.35	47.60	345.9	280.0	816.0	240.8	234.0	247.0
B	-2019	-1949	-2176	-68.20	-68.20	-71.57	-382.6	-338.8	-839.0	-347.6	-326.0	-353.7
C	-1.754	-2.569	-0.0916	0.0330	0.0330	0.0641	-1.648	-1.367	-4.780	-0.0775	-0.1303	0.1343
R		0.9164			0.8839		0.9637	0.9468	0.9809		0.9028	
SEM[2]		78.30			2.670			36.70			15.50	
R2[3]		91.90			89.50			67.70			87.70	

[1]C- control, formulated to meet the nutritional recommendation of Lohmann-LSL guide-line; L- 20% reduction of BP from C; and H- 20% increase of BP from C.
[2]Standard error of the mean.
[3]Coefficient of determination.

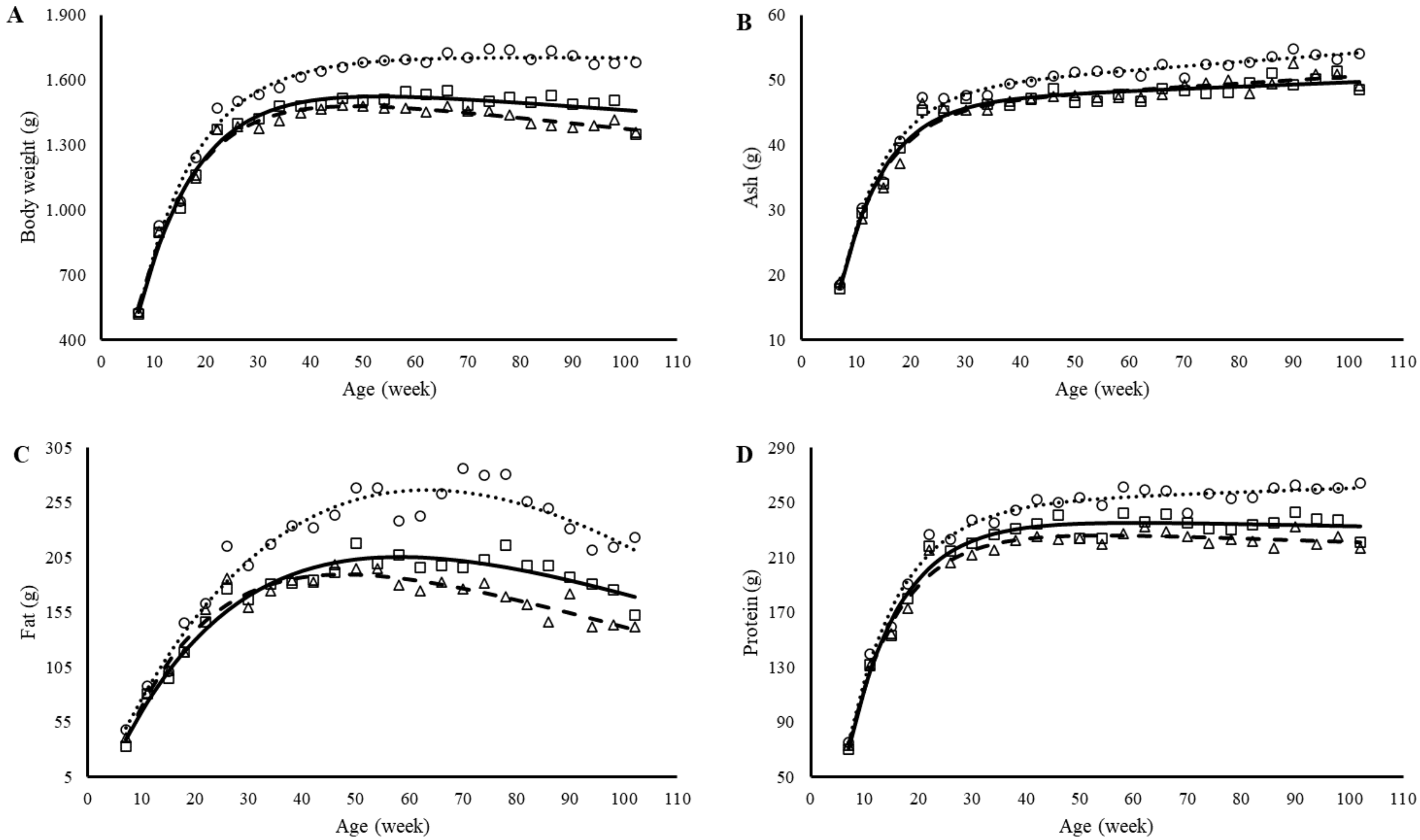

Figure 2. Observed and predicted body weight (A) and body components (ash (B), fat (C), and protein (D)) of laying hens from 8 to 102 weeks old fed three balanced protein feeds: control (□, —); low (Δ, - -) and high (○, ···).

Table 8. Egg weight and egg components of laying hens from 19 to 102 weeks old in response to three dietary balanced protein feeds.

Age,	Egg Weight, g					Yolk, %					Albumen, %					Shell, %				
weeks	C[1]	L	H	SEM[2]	P-value	C	L	H	SEM	P-value	C	L	H	SEM	P-value	C	L	H	SEM	P-value
22	51.2	51.3	51.9	0.590	0.7920	21.6	20.9	21.5	0.280	0.2160	67.2	68.1	67.2	0.320	0.1280	11.2	11.0	11.3	0.100	0.2160
26	57.9	55.5	59.4	0.590	0.0010	24.0	23.9	24.3	0.230	0.5230	65.6	65.6	65.2	0.280	0.5660	10.5	10.5	10.5	0.100	0.9710
30	59.3	58.5	60.4	0.590	0.1710	25.1	24.5	25.3	0.200	0.0320	64.5	65.4	64.6	0.220	0.0250	10.4	10.1	10.1	0.100	0.0780
34	61.1	59.7	61.7	0.590	0.1370	25.6	25.5	25.4	0.180	0.8880	64.1	64.3	64.4	0.200	0.6040	10.3	10.2	10.2	0.100	0.3500
38	61.9	60.6	62.2	0.590	0.2640	26.2	26.2	26.3	0.220	0.9790	63.6	63.5	63.5	0.220	0.9890	10.2	10.3	10.2	0.100	0.6580
42	62.4	61.4	63.6	0.590	0.1130	26.5	26.0	26.7	0.230	0.1380	63.6	64.1	63.6	0.250	0.4150	9.91	9.92	9.67	0.100	0.0630
46	62.2	60.2	63.4	0.590	0.0080	27.1	26.5	26.9	0.200	0.1570	63.0	63.5	63.3	0.220	0.3620	9.94	10.1	9.87	0.100	0.1510
50	63.4	60.3	63.7	0.590	0.0010	27.4	27.0	27.0	0.220	0.3500	63.0	63.3	63.4	0.230	0.6030	9.59	9.73	9.58	0.100	0.3610
54	63.8	62.6	64.7	0.590	0.1210	27.4	26.9	27.1	0.210	0.2960	62.8	63.4	63.3	0.230	0.2690	9.74	9.71	9.64	0.100	0.6010
58	63.1	62.1	64.2	0.590	0.1090	27.3	26.6	27.0	0.200	0.0950	63.1	63.8	63.4	0.210	0.1160	9.63	9.65	9.65	0.100	0.9820
62	65.6	64.4	65.3	0.590	0.4560	27.3	26.9	27.5	0.250	0.3580	63.2	63.7	63.1	0.270	0.2490	9.54	9.36	9.46	0.100	0.5010
66	66.6	64.9	66.2	0.590	0.2390	27.6	27.2	27.8	0.260	0.2260	62.9	63.4	62.6	0.310	0.2880	9.46	9.49	9.54	0.100	0.8390
70	65.9	63.0	66.5	0.590	0.0010	27.6	26.4	27.8	0.320	0.0050	63.0	63.8	62.7	0.370	0.1240	9.41	9.45	9.49	0.100	0.8170
74	66.4	62.4	66.1	0.590	<.0001	27.3	27.2	27.8	0.210	0.2200	63.4	63.4	63.0	0.230	0.4200	9.28	9.43	9.26	0.100	0.4160
78	66.2	62.0	67.5	0.590	<.0001	27.0	26.6	27.4	0.220	0.0830	63.7	64.1	63.5	0.250	0.2890	9.32	9.28	9.08	0.100	0.2300
82	65.7	61.9	65.5	0.630	0.0000	27.1	27.1	27.7	0.230	0.1250	63.8	63.7	63.1	0.240	0.1560	9.13	9.21	9.15	0.100	0.8210
86	65.5	61.6	66.1	0.590	<.0001	27.5	27.1	27.7	0.250	0.2540	63.5	63.8	63.3	0.260	0.3130	9.06	9.10	9.09	0.100	0.9700
90	66.9	61.3	67.6	0.590	<.0001	27.0	26.1	27.5	0.330	0.0200	64.1	64.7	63.5	0.310	0.0400	8.99	9.2	9.06	0.100	0.3330
94	66.9	61.4	65.6	0.630	<.0001	26.7	26.2	27.1	0.260	0.0910	64.5	64.7	63.9	0.310	0.1980	8.81	9.15	9.05	0.100	0.0980
98	66.9	62.1	66.8	0.590	<.0001	26.8	26.3	27.1	0.260	0.1400	64.5	65.2	64.1	0.340	0.0790	8.72	8.81	8.85	0.100	0.7340
102	65.8	63.9	66.2	0.590	0.0620	27.1	26.7	27.6	0.310	0.1360	64.1	64.5	63.5	0.370	0.2020	8.79	8.76	8.79	0.110	0.9800
Main effects																				
Age					<.0001					<.0001					<.0001					<.0001
Balanced Protein					0.0010					0.0600					0.1010					0.8480
Interaction					<.0001					0.0590					0.0970					0.1690

[1]C- control, formulated to meet the nutritional recommendation of Lohmann-LSL guide-line; L- 20% reduction of BP from C; and H- 20% increase of BP from C.
[2]Standard error of the mean.

Table 9. Coefficients for exponential equation for egg weight and egg components of laying hens from 19 to 102 weeks old in response to dietary balanced protein (BP).

Parameters	Egg Weight, g			Yolk, %			Albumen, %			Shell, %		
	C[1]	L	C	C	L	H	C	L	H	C	L	H
A1	66.60	62.50	66.40		27.10			63.6			8.23	
B1	-45.60	-115.0	-56.20		-81.10			234.0			3.72	
R	0.9450	0.8990	0.9340		0.8860			0.8300			0.9830	
SEM[2]		1.840			0.6540			0.7650			0.2030	
R2[3]		72.00			72.20			43.50			86.50	

[1]C- control, formulated to meet the nutritional recommendation of Lohmann-LSL guide-line; L- 20% reduction of BP from C; and H- 20% increase of BP from C.
[2]Standard error of the mean.
[3]Coefficient of determination.

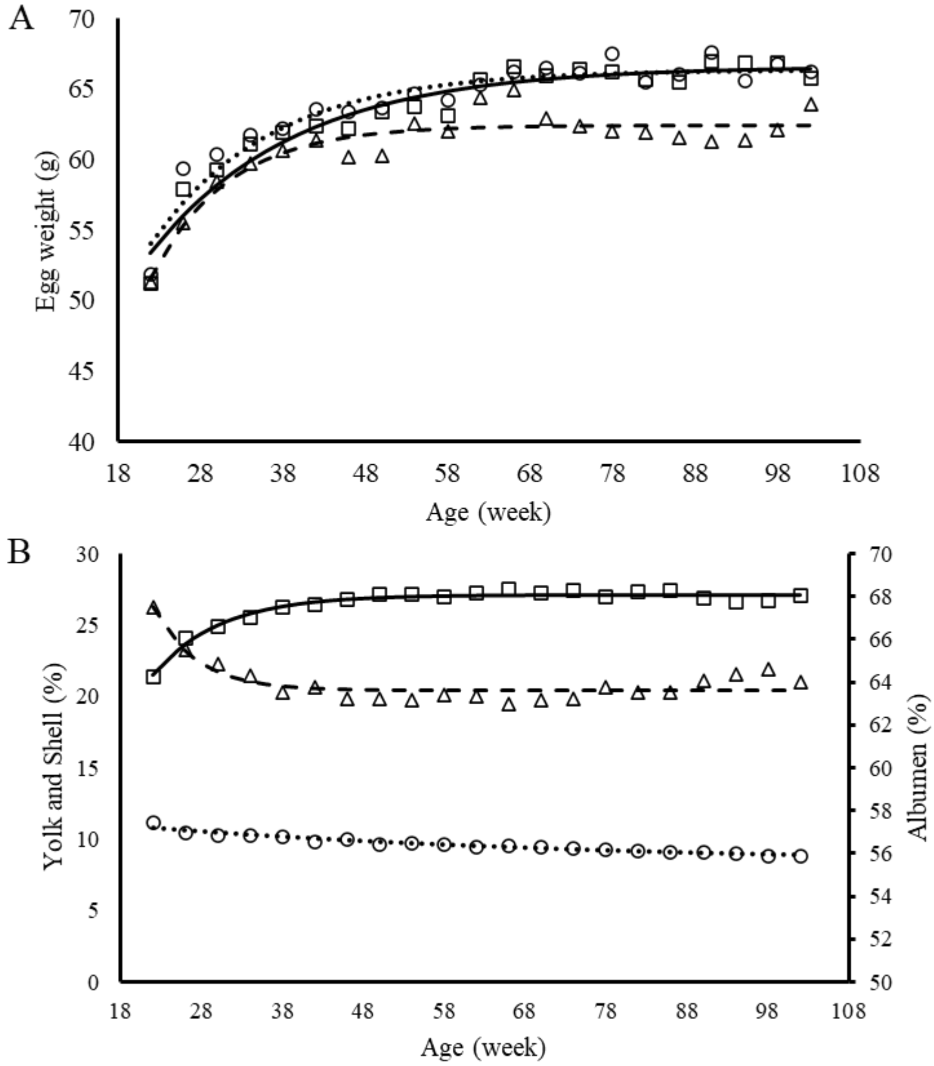

Figure 3. Observed and predicted egg weight (A) of laying hens from 19 to 102 weeks old in response to three dietary balanced protein feeds (control (□, —); low (Δ, - -), and high (○, ···)), and egg components (B): yolk (□, —), albumen (Δ, - -) and shell (○, ···).

CAPÍTULO 3 – Resposta de poedeiras à repleção e depleção de proteína balanceada na dieta

Este capítulo será apresentado de acordo com as normas da revista *Poultry Science*

Scientific section: Metabolism and Nutrition

Response of laying hens to repletion and depletion in dietary balanced protein

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Abstract

This study was carried out to evaluate the effects of dietary balanced protein (BP) at the growing phase and also to investigate the response of laying hens given a repletion or depletion of dietary BP during the laying phase period. At the beginning of the rearing period (eight wk-old), four-hundred pullets were equally distributed and received one of two experimental feeds: 1- Low BP (L); and 2- High BP (H). For the laying period (19 to 102 wk-old), four feeding program were designed based on the same treatments for rearing phases (LL, HH, LH, HL), where subsequent letters indicate the feed received on the rearing and laying period, respectively. Feed intake, body weight, and body composition were recorded during the rearing phases. The performance responses, egg quality, and body composition were periodically collected during the laying period. The data collected in the rearing phase (8 to 18 wk-old) were subjected to One-Way ANOVA, whereas data from the laying period was evaluated using a two-factor repeated measures analysis. Nonlinear regression models with groups were used to compare treatments in the laying phase, with the treatments being the group evaluated. In the rearing period, there was no difference in feed intake between pullets consuming feeds with different levels of dietary BP ($P>0.05$). Pullets consuming the feed H demonstrated higher body weight compared to treatment L ($P<0.05$). The age at sexual maturity was influenced by the BP in the rearing phase ($P<0.05$). In the first seven weeks of the laying period, feed intake was affected by dietary BP, but the average for the whole trial was similar between groups ($P>0.05$). All performance traits were somehow influenced by the level of BP in the feed ($P<0.05$). Hens subjected to the repletion treatment (LH) demonstrate a recovery in performance after the first feeding phase. The opposite result was observed for hens on the depletion treatment (HL). All egg components were affected by dietary BP ($P<0.05$), in which yolk was more effected than other egg components. The main difference in body composition is observed between hens consuming the LL and LH feeds, where the former demonstrates a higher concentration of body fat ($P<0.05$) and lower values for body protein ($P<0.05$). It was concluded that dietary balanced protein reduction affects the body composition of pullets, impacts long-term performance and egg components. It was demonstrated that a repletion of dietary balanced protein was efficient to recover the egg production performance of laying hens; although, the persistence of egg production was slightly better for the group of hens consuming a high dietary balanced protein in both rearing and laying phases.

Keywords: Body composition, egg components, egg production, ideal protein, laying hens

Introduction

The growing period of a laying hen is the most critical time of a hen's life and the mistakes made during this period are difficult to rectify (Leeson e Summer, 1981). Many factors, e.g., quantitative or qualitative feed restriction (Hussein, 2002; Saffar and Rose, 2007), feed program (Cheng, 1990; Summers, Leeson, and Spratt, 1987), and nutritional imbalance (Cheng et al., 1992; Silva et al., 2021), in the starter, grower and/or developer phases, have been reported to affect the growth curve, early egg weight and sexual maturity of pullets and consequently egg production. Therefore, genetic potential can only be achieved when the bird is provided with all its nutritional requirements (Thiele, 2012; Pottguter, 2016), especially when the objective is to extend the productive life of laying hens.

During the pullet rearing period, the focus is mainly on managing pullet body weight and body weight uniformity. However, current pullet feeding programs can lead to pullets of similar body weight, but with markedly different body compositions, which may affect life production (Bouvarel et al., 2011). Advances in genetic selection produced pullets quite different from those of only a few years ago. Despite many studies on feeding programs for egg-type hens during the rearing and lay period, information is still needed on combined feeding strategies between both periods in modern lines of hens.

The effects of dietary balanced protein for hens in the rearing phase and its impact on the long-term laying cycle were not investigated so far. The ideal supply of digestible amino acids during pullet formation is essential to ensure growth of organs, muscles, and skeleton (Leeson and Summers, 1997), while in the productive period, this contribution is essential for body maintenance and for the development of egg components (Bregendahl et al. 2008). Thus, the lack of balance of essential amino acids in the diet can affect pullet formation and its performance in the laying phase (Bregendahl et al., 2002; Babiker et al., 2010). In this context, we hypothesize that balanced protein levels will affect pullet formation, leading to a shift in the long-term laying cycle and the repletion in dietary balanced protein may recover the responses of laying hens; thus, the aim of the present research is to evaluate the impact of depletion and repletion of dietary balanced protein on body composition, performance, and egg quality in laying hens submitted to low and high protein nutrition during the rearing period.

Materials and Methods

Ethics Approval

All procedures described were approved by the Ethical Committee on the Use of Animals of the School of Agrarian and Veterinary Sciences, São Paulo State University (UNESP), Jaboticabal, São Paulo, Brazil (Process 012598/2018; approved on 14 February 2019).

Birds, husbandry, and experimental design

Four hundred, Lohmann LITE LSL-NA were obtained from a local commercial facility (Planalto Postura LTDA) at one day old and raised in conventional cages according to genetic guideline recommendations prior to the beginning of the trial. At eight weeks of age, pullets were moved to wire-rearing cages (375 cm^2 per pullet) and moved again at 19 weeks of age to wire-laying cages (563 cm^2 per hen). Each cage was equipped with a feeder and nipple drinker. Temperature, humidity, and lighting were maintained according to the recommendation of Lohmann LSL-NA Management Manual (Lohmann Tierzucht GmbH, Cuxhaven, Germany).

At the start of the trial (eight weeks of age), four hundred pullets were individually weighed (0.592 ± 0.012 kg) and moved to 20 cages to which two treatments were randomly assigned, performing ten replicates of 20 pullets each. At 19 weeks of age, each treatment was separated in two, giving a total of four treatments randomly distributed in five replicates each. During each experimental period, water and feed were provided *ad* *libitum*. The lighting program was set at 24L at the first week, reduced gradually to 12L:12D up to 10 weeks of age, and maintained until the pullets achieved 5% of egg production (20 weeks of age). After the onset of egg production, the lighting program was gradually increased from 12 to 16 h of light and kept constant up to 102 weeks of age.

A three-phase feeding program was used in the rearing period: grower (8-11 wk), developer (12-15 wk), and pre-layer (16-18 wk), while a five-phase feeding program was used for the laying period: Layer 1 (19 to 26 wk-old), Layer 2 (27 to 46 wk-old), Layer 3 (47 to 66 wk-old), Layer 4 (67 to 82 wk-old), and Layer 5 (83 to 102 wk-old).

Experimental feeds

Experimental feeds consisted of two levels of dietary balanced protein, herein named low (L) and high (H). Dietary balanced protein was defined as a constant ratio of essential amino acids with lysine (Eits et al., 2005) and the ratio was the same proposed by the breeding company (Lohmann Tierzucht GmbH, Cuxhaven, Germany). Standardized ileal digestible lysine (SID-Lys) was used as a reference to produce the two levels of dietary balanced protein. The remaining nutrients and energy in the feed were as recommended by the guideline (Lohmann-LSL Lite NA Management Guide 2019).

The grower, developer, and pre-layer feeds contained respectively 0.65, 0.56, and 0.59% of SID-Lys for L feeds and 0.96, 0.84, and 0.89% of SID-Lys for H feeds (Table 1). In the laying period (from 19 to 102 weeks of age), half replications continued receiving the L or H dietary balanced protein feeds (LL and HH), and the other half was submitted to repletion (LH) or depletion (HL), where subsequent letters indicate the feed supplied on rearing and laying phases, respectively. In the layer period, each one of the five feeds contained respectively 0.54, 0.52, 0,50, 0.48, and 0.46% of SID-Lys for L feeds and 0.82, 0.79, 0.75, 0.73, and 0.70% of SID-Lys for H feeds (Table 2).

Performance data

In the rearing period, cumulated feed intake and body weight were determined at 18 weeks of age. Mortality was registered daily and used to correct the feed intake. During the laying phase, egg production and mortality were recorded daily. Once a week, all eggs produced in one day were weighed and the egg mass was calculated. Feed intake was determined fortnightly. The age at sexual maturity was determined for each experimental unit and was defined as the age at 50% of egg production. Hen-housed egg production was calculated as the total number of eggs produced per number of housed hens at 19 wk-old.

Body composition

Laying hens selected at the beginning of the trial were individually scanned using dual-energy X-ray absorptiometry (DXA, Hologic-QDR® model 13.4.2., Marlborough, USA). In the rearing phase, DXA measurement was performed on the last day of each feeding phase, whereas in the laying phase measurements were taken every 28 days. The same birds were scanned over time. For that, a total of 16 pullets per treatment were used in the rearing period and 8 hens per treatment in the laying phase. Prior to each scan, hens were fasted for five hours, weighed, anesthetized with isoflurane (2%) diluted in 100% of oxygen, and positioned in dorsal decubitus with the wings and legs flexed (Alves et al., 2019). The fat mass (g), lean mass (water + protein content, g), bone mineral content (g), and bone mineral density (g/cm^2) were registered. Alves et al. (2019) equation was used to estimate the ash, fat and protein content in g/100g of body weight.

Egg traits and egg components measurement

Every four weeks, three eggs per experimental unit were sampled in three sequential days, totaling nine eggs per experimental unit. The eggs were individually weighed and numbered. The egg components, albumen, yolk, and dry eggshell were measured. Before measurement, eggshell was washed with tap water and dried using a forced oven at 55°C for 24 hours. Additionally, the strength and shell thickness were analyzed using the Nabel Digital Egg Tester 6000® (Kyoto, Japan).

Statistical analysis

The feed intake, body weight, and body composition measured during the rearing period were analyzed as One-Way ANOVA, using a generalized linear model. In the laying phase, the age at sexual maturity and hen-housed egg production were evaluated as One-Way ANOVA and other responses were evaluated as a two-factor repeated measure design to determine the effects of dietary treatments over time, using a mixed model. One factor is represented by the four treatment groups (LL, LH, HH, and HL) and the other factor is the age of hens. The data was analyzed considering 21 cycles of four weeks each. Orthogonal contrasts were elaborated to investigate the effects of repletion (LL vs LH) and depletion (HH vs HL) of dietary balanced protein. Differences were considered to be significant at a probability of 5%. The Statistical Analysis System (SAS Institute Inc., Cary, NC) was used to perform both a One-Way ANOVA and the two-factor repeated measure analysis procedures.

To investigate how the responses differed between treatment groups over time, non-linear regression with groups was used, the groups being the dietary balanced protein (GENSTAT, VSN International 2017). The average data per replicate were treated as the experimental unit. Two exponential models were applied and that with the lower Akaike information criterion value (AIC; Akaike, 1974) was used to describe the response variable. The models used were:

Linear plus exponential: $y = A + B * (R^x) + C * x$

where A and C are the y-intercept and slope of the linear segment respectively, B is the y-intercept of the exponential segment, R is the exponential base, and x is the age in weeks.

Exponential: $y = A1 + B1 * (R1^x)$

where A1 + B1 is the y-intercept, R1 is the exponential base, and x is the age in weeks.

Results

Rearing period

Performance parameters and body composition of pullets for rearing phase are shown in Table 3. The dietary balanced protein affected the body weight (P<0.05) but have no effect on cumulated feed intake (P>0.005). Even with a similar cumulated feed intake between groups, pullets consuming the feed with a higher level of balanced protein were about 3% heavier at the end of rearing period (18 weeks of age). The observed results indicate that pullets fed L feed were not able to consume a sufficient amount of protein to support the growth, reducing the body weight gain during the rearing period. The dietary balanced protein did not affect the body composition evaluated (P>0.05).

Laying period

Performance

The age at sexual maturity was statistically different between groups (Table 4). Increasing the dietary balanced protein about one week before the onset of lay did not change the age at sexual maturity (LL vs LH, P>0.05) and a similar response is observed when a decrease in dietary balanced protein is applied (HH vs HL, $P>0.05$). On the other hand, the feed offered in the rearing phase influenced the age at sexual maturity ($P<0.05$). Laying hens consuming the L feed had seven days of delay in the age at sexual maturity in comparison with hens consuming the H feed. Alike, the reduction of dietary balanced protein in the rearing period affected the hen-housed egg production ($P<0.01$). On average, laying hens given the L feed in the rearing phase reduced the egg production in 30 units in comparison with hens consuming the H feed; however, the repletion or depletion of dietary balanced protein did not affect this response variable ($P>0.05$).

The interactions for the two-way repeated measure were significant for all performance responses evaluated in the laying period (Table 5). As a consequence of the feed given in the previous phase, feed intake was different between groups during the first six weeks after the onset of lay. When laying hens consumed the H feed in the rearing period, they increased their feed intake in a higher ratio compared with hens from the L group (Supplementary Table 1). These difference, however, was not consistent over the time and the mean feed intake accounted for the whole laying phase was similar between groups ($P>0.05$). The non-linear regression with groups applied for feed intake in function of age (Table 6) indicates that three parameters were affected by treatment and only the parameter R was similar between groups. On average, the repletion of dietary balanced protein improved (LL vs LH, $P<0.05$) egg production (3.7 %), egg weight (3 g), egg mass (4.9 g), feed conversion (0.16 g/g), and increased mean body weight (153 g). A depletion of dietary balanced protein reduced (HH vs HL, $P<0.05$) egg weight (-3.5 g) and egg mass (-4.5 g) and increased feed conversion (0.18 g/g) of laying hens. The regression with groups (Table 6) demonstrates that the performance responses of laying hens were affected by treatment and only the exponential base (R) of the equation was similar for all treatments indicating a similar behavior between groups but different ratios and maximum/minimum estimates. The peak of egg production was estimated at 30 weeks of age for all treatment groups; however, the repletion of dietary balanced protein seems to recover the egg production rate at the peak (98%) in comparison with hens in the LL group (95%). On the contrary, there is a difference of two weeks in the peak in egg mass between hens from LL and LH groups, in which the former increased 4g in egg mass at the peak (Figure 1). The depletion of balanced protein (HH and HL) affected the peak of egg mass for about one week. These results can also be observed in the supplementary Table 2.

Body composition

There was an interaction between treatment and age of laying hens for ash, fat, and protein contents in the body ($P<0.05$, Table 5 and Supplementary Table 3). The results suggest that the differences are mainly due to the group of hens fed with the LL feeds (Figure 2). On average, hens given a repletion of dietary balanced protein increased 2.4 percentual points in the fat content compared with hens in the LL group but reduced by 0.36 and 0.70 percentual points in the contents of ash and protein in the body ($P<0.05$). The regression between groups indicate that both dietary balanced protein and the repletion/depletion treatments affected the dynamics of body composition over time ($P<0.05$).

Egg quality

Overall, the interaction between treatment and the age of hens was statistically different for all egg components and eggshell strength (Table 5 and Supplementary Table 4). Laying hens consuming the LH and HH feed produced eggs with heavier yolk in comparison with hens consuming the LL or HL feeds (around 6% difference). The results indicate that the feed given in the rearing phase has a limited influence on the yolk production. The differences observed for albumen and eggshell weights suggest a similar behavior. The heavier albumen and eggshell were produced by hens consuming a feed with a higher balanced protein level (P<0.05), disregarding the feed given in the rearing phase. The differences in eggshell strength were evident for the depleted treatment (HH vs HL, P<0.05), reducing the eggshell strength by about 3%. The eggshell thickness was similar between treatments (P>0.05). The regression with groups demonstrate that individual equations are necessary to predict the egg components and eggshell strength over time (Table 5 and Supplementary Table 5).

Discussion

The nutrition given to laying hens in the rearing phase may influence the growth and consequently their degree of body maturity. However, it is well known that sexual maturity is most influenced by photoperiod, with body weight having a minor effect (Lewis and Morris, 2006), opening an opportunity to change the pullet's nutrition without affecting the sexual maturity, but hens response over long-term egg production needs to be investigated. The objective herein was to evaluate laying hens regarding the effect of dietary balanced protein given in the rearing phase and how they respond to a repletion or depletion of dietary balanced protein in the laying phase. We hypothesized that offering a low dietary balanced protein feed to pullets from 8 to 18 weeks of age would produce a lighter hen with, perhaps, higher body fat content when compared with a hen consuming a high balanced protein feed. Those differences would have a minimum impact on the age at sexual maturity but the low dietary balanced protein feed would not be sufficient to sustain a high egg production or egg mass. An even more interesting question to be answered is whether those effects are reversible if the dietary balanced protein is repleted in the laying phase.

In this study, the age at sexual maturity (50% of egg production) was influenced by the feed given in the rearing phase, where pullets in the higher dietary balanced protein feed reached sexual maturity about 7 days before, which may have elicited an increase on feed intake prior to the laying hens consuming the L feed, minimizing the difference on cumulated feed intake at the end of rearing phase (18 weeks of age). In fact, the results published elsewhere by Nobrega et al. (2022), demonstrate an increase in feed intake due to a reduction in dietary balanced protein, which is minimized when pullets approach 15 weeks of age. According to Bendezu et al. (2018), the development of the ovary and oviduct is maximized around 15 to 16 weeks of age, which affect the needs for energy and nutrients, consequently, feed intake. Body weight, on the other hand, was clearly affected by dietary balanced protein, with no effect on body composition. Those results indicate that pullets from distinct groups were at a different degree of body maturity, which may also influence the age at sexual maturity. Lewis and Morris (2006), evidenced that laying hens maintained in the same photoperiod but with different body weight achieved the onset of lay and the age at sexual maturity on different days, corroborating with our observations. Although, the authors highlighted that the photoperiod has much more influence over the onset of lay.

The rate of sexual maturation is coordinated by hormones such as luteinizing hormone (*LH*) and follicle stimulating hormone (*FSH*), produced in the pituitary gland (Du et al., 2020). The release of *LH* and *FSH* is stimulated by the gonadotropin releasing hormone (*GnRH*), produced in the hypothalamus (SCANES, 1984). The former is also referred to as extra-retinal or deep encephalic photoreceptor, since light perceived in such region of the brain will control the secretion of *GnRH*. A system called hypothalamo-hypophyseal-gonadal axis allows the *GnRH* to reach the pituitary gland and initiate the release of *LH* and *FSH* (Du et al., 2020). Another hormone that also controls the *LH* and

FSH release is the gonadotropin inhibitory hormone (*GnIH*). The *GnIH*, also produced in the hypothalamus is antagonistic to *GnRH* and will prevent the pituitary to release the *LH* and *FSH* hormones (Ciccone et al., 2004). Both *GnRH* and *GnIH* are peptide hormones thus requiring a receptor in the site of action to bring about its function. The *GnIH* receptors in the pituitary are reported to decrease in Lohmann hens between 17 to 20 weeks of age, while the *GnRH* receptors increase at the same age (Hanlon et al., 2021). Those events may increase the release of *LH* and *FSH* and contribute to the onset of lay. A possible explanation for the shift in the age at sexual maturity for hens submitted to the same photostimulation but consuming different levels of dietary balanced protein is that a delay in the degree of body maturity observed in hens consuming the L feed may also delay the changes in *GnRH* and *GnIH* receptors in the pituitary gland, but this hypothesis needs to be tested.

The objective to produce different laying hens at the end of rearing phase was achieved but the body composition was the similar between groups. The effect of dietary balanced protein over body fat is well documented in the literature for broilers and breeders (Azevedo et al., 2021; Van Emous et al., 2015). Those studies report that body fat percentage increases with the reduction of dietary balanced protein. Our results demonstrate that body fat content was similar between treatment groups at the end of rearing phase, showing a different trend from the ones reported for broiler and breeder. One may expect that reducing dietary balanced protein would reduce the amount of protein available for deposition and, hence, the energy once used for protein deposition would be available for lipid deposition. These events become especially true if the feed intake is maintained constant or increases with the reduction of dietary balanced protein. However, our observations suggest that this is not to be the case for growing pullets after eight weeks of age. The degree of body maturity may, again, be one possible explanation.

The reduction in dietary balanced protein delays the body protein deposition and, perhaps, the development of reproductive organs in laying hens. As the hens approach their sexual maturity, the development ratio of the ovary and oviduct increases rapidly, and lipid deposition in the ovary contributes mostly to such an increase (Bendezu et al., 2018). Since laying hens in the H group was advanced in body development, their ovary and oviduct development may have started earlier, when compared with hens in the L group, increasing the lipid deposition in the body and minimizing the differences from pullets consuming the L feed.

Once in production, it is useful to know if the consequences of giving a low protein feed in the rearing phase can be reversed. For that, a repletion treatment was included in the treatment design. The overall results demonstrate that repleted hens (LH) increase all responses evaluated, with an exception for daily feed intake and eggshell traits. The data presented (Table 5) demonstrate that repletion of dietary balanced protein could be a strategy to recover a pullet that reaches sexual maturity with low body weight. In addition, there may be an economic benefit to reduce balanced protein in the feed because the feed price would rather decrease (Azevedo et al., 2021). Since feed intake was similar between groups, the feeding cost (feed intake x feed price) would also reduce. The egg mass was similar between hens consuming the H feed in the laying phase (LH and HH), suggesting that the revenue obtained from either group of hens would be the same. Nevertheless, an economic investigation is necessary to better understand this issue, which was not the goal of this study. Another issue that is worth investigating is related to the effects of depletion of dietary balanced protein. The change in feed ingredients price may trigger nutritionists to reduce the price of a feed formula, sometimes by reducing dietary balanced protein level. To properly evaluate, the laying hens response due to a reduction in dietary balanced protein, the current status of the bird needs to be accounted for.

We showed herein that laying hens receiving high dietary balanced protein feed in the rearing phase were able to increase feed intake at the beginning of egg production when dietary balanced protein was depleted. As a result, this group of birds had the highest lipid content in the body, even though, on average such difference was not statistically different from hens in the HH group. Possible because to recover the amount of dietary balanced protein that was removed from feed, laying hens would need to increase the feed intake about 40%, which was, perhaps, beyond the intestinal bulk capacity of these hens. In this study, to reduce dietary balanced protein, it was necessary to include more wheat brand in the feed compared with other treatments. That might have limited the bulk capacity of the gastrointestinal tract, constraining the feed intake. Recently, Nascimento et al. (2021) demonstrated that broiler breeders could increase their feed intake as the feed is diluted to achieve their nutrients and energy needs, but the intake of feed decrease at a higher dilution.

An interesting data produced in this study is the time necessary to change the response of laying hens when a repletion or a depletion feed is offered. According to the repeated measures analysis, it took 11 weeks to detect a difference in egg production between groups, while for egg weight and egg mass seven weeks after the beginning of the repletion and depletion treatments were necessary to affect those variables. The nonlinear regression also indicates that the ratio of increase for each mentioned variable was different, which is demonstrated in figure 2. A decrease in egg production, egg weight, and egg mass is reported in laying hens consuming crescent levels of dietary balanced from 26 to 77 weeks of age (Kumar et al., 2018). The pattern of body chemical components over time changed consistently after 50 weeks of age, especially for body fat. Laying hens in the LL group demonstrate the lowest body fat content compared to the other treatments. The reduction in body fat content for laying hens consuming a low dietary balanced protein feed was not expected; however, Kumar et al. (2018), found a quadratic response in abdominal fat in function of dietary balanced protein concentration. In the present study, the results from repletion and depletion groups might require a separate interpretation. When compared with hens from LL group, the higher value of body fat content observed in repleted hens might be related to the lipid content in the ovarium, since those hens produced eggs with heavier yolk. On the other hand, compared with HH group, depleted hens increased body fat deposition possibly due to an increase in feed intake during the first weeks after the depleted feed was offered, increasing energy intake. On either situation, any conclusion over the dynamics of body fat content in laying hens should be carefully evaluated, and more studies are necessary to better elucidate this response.

In this study, the results observed for egg production and egg weight suggest that the feed offered in the rearing phase has a low influence on those responses. The hen-housed egg production, however, was influenced only by the feed offered in the rearing phase (LL+LH vs HH+HL). The observed differences might be a consequence of the viability observed during the trial. The viability of laying hens consuming the L feed during the rearing phase was 87.5% whereas hens consuming the H feed had a viability of 90%. Grossman et al., (2000), suggest that hens with similar total production may have diferente egg production curve, mainly due to persistency. The persistency in egg production is defined as the decline ratio observed over time (Flock, 1980; Muir 1990). In this study, the parameter C in the equation adjusted for egg production in function of time is related to a declining ratio after the maximum point (peak of egg production). The results indicate that laying hens in the LL group reduced the egg producion after peak faster than the other groups, followed by HH, LH, and HL.

According to Bregendahl et al. (2008), the metabolic fate of dietary protein in the laying phase is mainly to support oocytes and follicle growth; the depletion of dietary balance protein significantly impacts the reproductive response. Likewise, Kumar et al. (2018), a deficiency of amino acid involves a mandatory muscle tissue catabolism to sustain the maintenance and reproductive functions (Hurwith and Bornstein, 1973).

Conclusion

The results presented herein demonstrate how pullets respond to dietary balanced protein and the consequences of a repletion or a depletion of dietary balanced protein in the laying phase. The adverse effects of reducing the balanced protein in the growing phase were minimized by repleting the dietary balanced protein in the laying period. On the other hand, depletion of balanced protein in the layer phase reduced the performance of hens, reaching similar results of hens consuming the lower protein diet during the whole study.

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Declaration of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Software and data repository resources

None of the data was deposited in an official repository. The data can be obtained from the authors upon request.

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Table 3. Composition and calculated nutritional content of experimental feeds in rearing period

Ingredients	8 to 11 weeks Low	8 to 11 weeks High	12 to 15 weeks Low	12 to 15 weeks High	16 to 18 weeks Low	16 to 18 weeks High
Corn (7.8%)	68.0	56.3	63.0	53.4	61.0	49.9
Soybean meal (45%)	15.0	31.6	10.0	23.8	10.0	24.2
Wheat bran	13.0	7.10	20.0	15.0	20.0	14.0
Potassium carbonate	0.240	-	0.210	-	0.280	0.005
Corn gluten (60%)	-	-	1.50	1.50	-	2.00
Meat and Bone Meal 48%	-	-	2.66	2.66	2.97	3.87
Soy oil	0.150	1.50	0.370	1.50	0.685	1.42
Dicalcium phosphate	1.17	1.20	0.140	0.161	0.210	-
Limestome	1.46	1.32	1.38	1.26	3.98	3.75
Salt	0.287	0.420	0.215	0.306	0.215	0.256
Sodium Bicarbonate	0.200	-	0.200	0.065	0.198	0.110
Vit. and Min. supplement[1]	0.200	0.200	0.200	0.200	0.200	0.200
DL-Methionine (99%)	0.055	0.161	-	0.114	0.045	0.111
L-Lysine HCl (78%)	0.100	0.024	0.038	-	0.095	0.027
L-Threonine (98.5%)	-	0.031	-	-	-	-
Choline chloride (60%)	0.100	0.100	0.100	0.100	0.100	0.100
Total	100	100	100	100	100	100
-------------------- Calculated nutritional content (%) --------------------						
Met. energy (kcal/kg)	2883	2880	2860	2860	2778	2778
Crude protein[2]	14.3	20.0	14.7	19.5	13.9	20.2
Dig. Lysine	0.645	0.960	0.560	0.840	0.593	0.890
Dig. Methionine + cysteine	0.476	0.709	0.434	0.653	0.442	0.662
Dig. Threonine	0.475	0.703	0.471	0.635	0.441	0.652
Dig. Tryptophan	0.148	0.226	0.139	0.203	0.133	0.206
Dig. Isoleucine	0.496	0.760	0.480	0.698	0.444	0.719
Dig. Valine	0.568	0.827	0.573	0.787	0.532	0.812
Calcium	1.04	1.04	1.05	1.05	2.08	2.08
Available Phosphurus	0.460	0.460	0.430	0.430	0.457	0.457
Sodium	0.180	0.180	0.170	0.170	0.170	0.170

[1]Content (per kg of product) Vit. A 4,850,00 Ul, Vit. D3 1,350,000 Ul, Vit. E 8,500 Ul, Vit. K3 1,395 mg, Vit. B1 1,000 mg, Vit. B2 2,570 mg, Pantothenic acid 5,295 mg, Vit. B6 1,525 mg, Vit. B12 7,500 mcg, Niacin 19.45 g, Folic acid 500 mg, Biotin 41.50 mg, Choline chloride 75 g, Iron 22 g, Copper 4,500 mg, Manganese 25 g, Zinc 25 g, iodine 500 mg, selenium 125 mg, Phytase 300,000 FYT.

[2]Values represent the mean analyzed composition by near-infrared spectroscopy (NIR).

Table 4. Composition and calculated nutritional content of experimental feeds in the laying phase

Ingredients	19 to 26 weeks		27 to 46 weeks		47 to 66 weeks		67 to 82 weeks		83 to 102 weeks	
	Low	High	Low	High	Low	High	Low	High	Low	High
Corn (7.8%)	65.0	53.0	67.0	55.3	67.5	56.2	68.5	58.0	68.7	58.1
Soybean meal (45%)	10.1	22.0	13.0	23.9	11.6	22.3	11.7	21.4	11.9	20.2
Wheat bran	6.00	1.49	4.70	-	5.00	-	5.00	-	5.00	-
Potassium carbonate	0.560	0.340	0.470	0.302	0.525	0.325	0.500	0.315	0.510	0.340
Corn gluten (60%)	5.00	10.0	2.55	8.30	2.95	8.56	1.95	8.07	1.10	7.98
Soy oil	0.890	1.25	0.610	0.840	0.570	0.790	0.580	0.552	0.660	0.680
Dicalcium phosphate	1.30	1.32	1.14	1.17	1.09	1.14	1.09	1.14	0.98	1.04
Limestome	9.17	9.06	9.51	9.41	9.82	9.72	9.82	9.72	10.4	10.3
Salt	0.279	0.336	0.290	0.356	0.275	0.310	0.260	0.280	0.280	0.270
Sodium Bicarbonate	0.200	0.110	0.183	0.080	0.168	0.110	0.190	0.157	0.160	0.172
Vit and Min supplement[1]	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200	0.200
DL-Methionine (99%)	0.041	0.090	0.047	0.082	0.036	0.068	0.038	0.059	0.038	0.050
L-Lysine HCl (78%)	0.119	0.078	0.029	-	0.038	-	0.022	-	-	-
Choline chloride (60%)	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100	0.100
Washed sand	1.023	0.621	0.150	-	0.159	0.171	0.052	-	-	0.495
Total	100	100	100	100	100	100	100	100	100	100
-------------------- Calculated nutritional content (%) --------------------										
Met. energy (kcal/kg)	2795	2795	2785	2785	2785	2785	2785	2785	2770	2770
Crude protein[2]	15.6	21.4	14.6	20.6	13.3	20.2	11.7	18.7	11.4	18.7
Crude Fibre	2.22	2.25	2.50	2.22	2.22	2.16	2.23	2.14	2.23	2.09
Dig. Lysine	0.544	0.816	0.524	0.786	0.500	0.750	0.484	0.726	0.464	0.696
Dig. Methionine + cysteine	0.480	0.720	0.464	0.696	0.448	0.672	0.432	0.648	0.416	0.624
Dig. Threonine	0.459	0.683	0.459	0.681	0.446	0.664	0.433	0.647	0.420	0.627
Dig. Tryptophan	0.123	0.192	0.131	0.196	0.124	0.188	0.123	0.183	0.121	0.176
Dig. Isoleucine	0.486	0.781	0.484	0.776	0.468	0.755	0.450	0.731	0.434	0.707
Dig. Valine	0.563	0.869	0.553	0.859	0.539	0.839	0.518	0.813	0.499	0.789
Calcium	3.95	3.95	4.05	4.05	4.15	4.15	4.15	4.15	4.35	4.35
Available Phosphurus	0.440	0.440	0.410	0.410	0.400	0.400	0.400	0.400	0.380	0.380
Sodium	0.175	0.175	0.175	0.175	0.165	0.165	0.165	0.165	0.165	0.165

[1]Content (per kg of product) Vit.. A 4,850,00 Ul, Vit.. D3 1,350,000 Ul, Vit. E 7,785 Ul, Vit. K3 1,195 mg, Vit. B1 1,200 mg, Vit. B2 3,000 mg, Pantothenic acid 4,236 mg, Vit. B6 1,522 mg, Vit. B12 7,708 mcg, Niacin 16.21 g, Folic acid 500 mg, Biotin 41.50 mg, Choline chloride 93.75 g, Iron 22 g, Copper 4,500 mg, Manganese 25 g, Zinc 25 g, iodine 500 mg, selenium 125 mg, Phytase 300,000 FYT.

[2]Values represent the mean analyzed composition by near-infrared spectroscopy (NIR).

Table 5. Performance and body composition of pullets fed levels of balanced protein (BP) at 18 weeks of age

Variables	Treatments[1]			
	L	H	SEM[2]	P-value[3]
Cumulative feed intake, g/bird	4,445	4,391	36.75	0.3418
Body weight, g/bird	1,203	1,249	10.4	0.0089
Ash, %	3.91	3.90	0.033	0.9581
Fat, %	12.7	13.2	0.257	0.1673
Protein, %	18.0	17.9	0.121	0.5421

[1]L and H, reduction and accretion 20% balanced protein (BP) from nutritional recommendations of Lohmann feeding program, respectively.

[2] SEM: Stander error of the mean.

[3]ANOVA at 5% probability level.

Table 6. Age at 50% production and hen house egg production of laying hens in response dietary balanced protein levels in development period and laying period

Treatments[1]	Age at 50% production	Hen house egg production
LL	147	485
LH	146	500
HH	140	529
HL	142	517
SEM	1.10	8.11
P-value	0.0007	0.0031
Ortogonal Contrasts		
LL vs. LH	0.5082	0.2118
HH vs. HL	0.2323	0.3293
(LL, LH) vs. (HH, HL)	<.0001	0.0006

[1]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
SEM: Stander error of the mean.

Table 7. Analysis of variance and contrasts for performance, body composition, and egg quality of laying hens from 19 to 102 weeks old in response to four diets program with distinct levels of dietary balanced protein feeds

	Treatments[1]					**Source of variation**			**Ortogonal Contrasts**	
Variables	LL	LH	HH	HL	SEM[2]	Cycles[3]	Treatments	Cycles × Treatments	LL vs. LH	HH vs. HL
---------- Performance ----------										
Feed intake, g/bird/day	107	107	108	109	1.56	***	n.s.	*	n.s.	n.s.
Egg production, %	86.3	90.0	91.6	89.7	1.33	***	***	***	**	†
Egg weight, g	60.3	63.3	63.6	60.1	0.619	***	***	***	***	***
Egg mass, g	52.4	57.3	58.4	53.9	1.01	***	***	***	***	***
Feed conversion ratio, g/g	2.17	2.01	1.90	2.08	0.029	***	***	***	***	***
Mean Body weight, g/hen	1,422	1,575	1,658	1,615	41.2	***	**	***	*	n.s.
---------- Mean Body composition (%) ----------										
Ash	3.93	3.57	3.55	3.65	0.073	***	***	***	***	n.s.
Fat	13.7	16.1	16.4	17.0	0.736	***	**	***	**	n.s.
Protein	18.1	17.4	17.5	17.0	0.263	***	**	**	*	†
---------- Mean Egg response ----------										
Yolk. g	15.9	16.8	17.1	15.9	0.250	***	***	***	**	***
Sell. g	5.86	6.04	6.12	5.83	0.082	***	**	***	*	***
Albumen. g	39.2	41.0	40.9	38.9	0.548	***	**	***	**	**
Shell strength, kgf	4.35	4.43	4.47	4.33	0.101	***	n.s.	*	n.s.	*
Shell thickness, mm	0.381	0.380	0.383	0.380	0.004	***	n.s.	†	n.s.	n.s.

[1]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.

[2]SEM: Stander error of the mean.

[3]Cycles: Every four weeks from 19-week-old.

*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, †P ≤ 0.10, and n.s. P > 0.10

Table 8. Regression models for performance of laying hens from 19 to 102 weeks old subjected to four feeding programs varying balanced protein levels. Models: line plus exponential, A + B × (R^{age}) + C × age; exponential asymptote, A1 + B1 × (R^{age})

Parameters	LL[1]	LH	HH	HL
		---------- Feed intake, g/bird/day ----------		
A	109.90	110.30	113.20	111.60
B	-2786	-2642	-2541	-2231
C	0.0020	-0.0014	-0.0376	-0.0110
R		0.8185		
SEM[2]		4.04		
R2[3]		75.0		
		---------- Egg production, % ----------		
A	101.60	103.20	103.90	101.90
B	-159459287	-159945455	-123767487	-138501947
C	-0.1890	-0.1535	-0.1556	-0.1486
R		0.5142		
SEM		2.80		
R2		95.5		
		---------- Egg weight, g ----------		
A1	62.060	65.510	65.770	61.650
B1	-139.60	-175.90	-167.30	-127.30
R		0.89758		
SEM		1.95		
R2		80.5		
		---------- Egg mass, g ----------		
A	60.430	62.520	63.490	60.940
B	-267264	-273987	-243149	-240045
C	-0.0858	-0.0384	-0.0431	-0.0740
R		0.6741		
SEM		2.65		
R2		92.3		
		---------- Feed conversion ratio, g/g ----------		
A	1.752	1.740	1.740	1.800
B	1856646742	121069894	121069894	1135513471
C	0.0039	0.0012	0.0012	0.0027
R	0.4019	0.4405	0.4405	0.4019
SEM		0.06		
R2		99.1		
		---------- Body weight, g----------		
A	3678.0	4842.0	4615.0	5028.0
B	-2485.0	-3818.0	-3454.0	-3876.0
C	-13.650	-17.910	-16.280	-19.710
R		0.9904		
SEM		103		
R2		52.10		

[1]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
[2]Standard error of the mean.
[3]Coefficient of determination.

Table 9. Coefficients of exponential equation for body weight and body composition of laying hens from 19 to 102 weeks old in response to four diets program with distinct levels of dietary balanced protein feeds. Model: line plus exponential, A + B × (R^{age}) + C × age

Parameters	LL[1]	LH	HH	HL
		---------- Ash, % ----------		
A	2.599	2.640	1.960	-3.100
B	2.801	1.500	2.736	7.500
C	0.0169	-0.0385	0.0161	0.0450
R	0.9554	1.0122	0.9723	0.9890
SEM[2]		0.154		
R2[3]		64.70		
		---------- Fat, % ----------		
A	1018.0	2119.0	2357.0	2845.0
B	-1006.0	-2110.0	-2348.0	-2836.0
C	1.417	3.045	3.383	4.072
R		1.0013		
SEM		1.65		
R2		51.20		
		---------- Protein, % ----------		
A	15.230	12.700	11.630	10.070
B	3.002	6.525	7.800	9.185
C	-0.0472	-0.1264	-0.1476	-0.1727
R		1.0101		
SEM		0.546		
R2		48.40		

[1]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
[2]Standard error of the mean.
[3]Coefficient of determination.

Table 10. Coefficients of exponential equation for egg response of laying hens from 19 to 102 weeks old in response to four diets program with distinct levels of dietary balanced protein feeds. Model: exponential asymptote, A1 + B1 × (R^{age})

Parameters	LL[1]	LH	HH	HL
		---------- Yolk weight, g ----------		
A1	16.695	17.867	18.189	16.585
B1	-80.000	-47.620	-41.640	-87.100
R	0.88839	0.91377	0.91873	0.88236
SEM[2]		0.645		
R2[3]		86.8		
		---------- Shell weight, g ----------		
A1	5.990	6.113	6.203	5.951
B1	-0.00154	-0.00083	-0.00095	-0.00145
R		1.0594		
SEM		0.21		
R2		40.2		
		---------- Albumen weight, g ----------		
A1	40.300	42.450	42.210	39.770
B1	-18.350	-25.020	-23.240	-15.600
R		0.9410		
SEM		1.47		
R2		59.5		
		---------- Shell strength, kgf ----------		
A1	-6.53	26.3	-5.23	103
B1	13.25	-19.9	11.9	-97
R	0.99677	1.0015	0.99664	1.00032
SEM		0.185		
R2		95		

[1]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
[2]Standard error of the mean.
[3]Coefficient of determination.

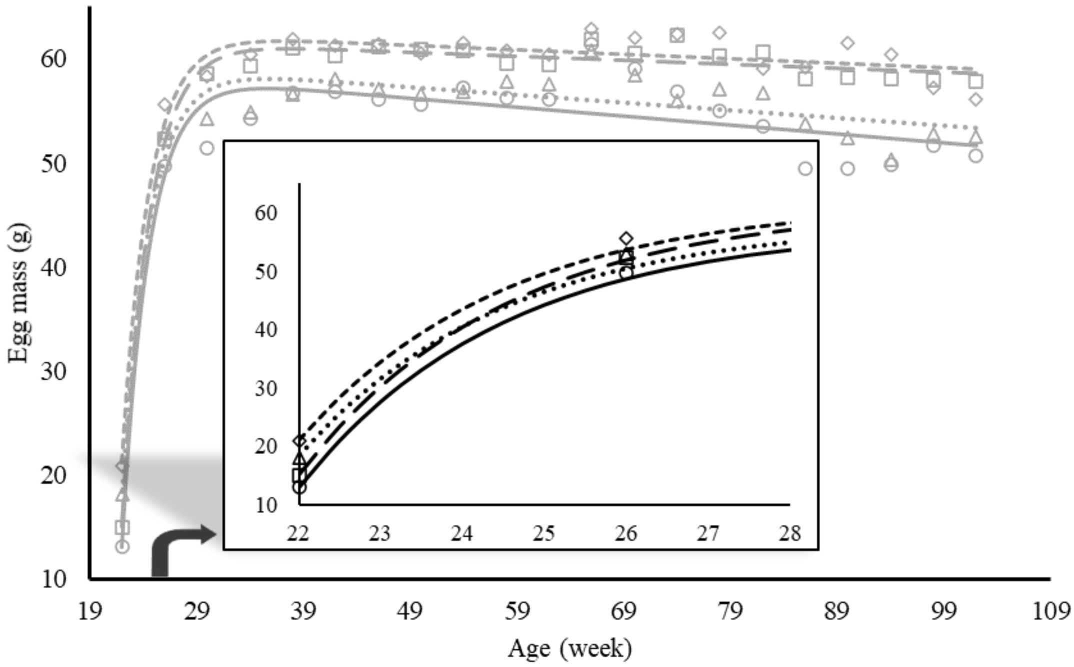

Figure 1. Observed and predicted egg mass (g) of laying hens from 19 to 102 weeks old in response to four diets program with distinct levels of dietary balanced protein feeds. Treatments: LL (○, —). and HH (◊, - -), reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH (□, – –) and HL (Δ, ···), repletion and depletion of BP in laying period

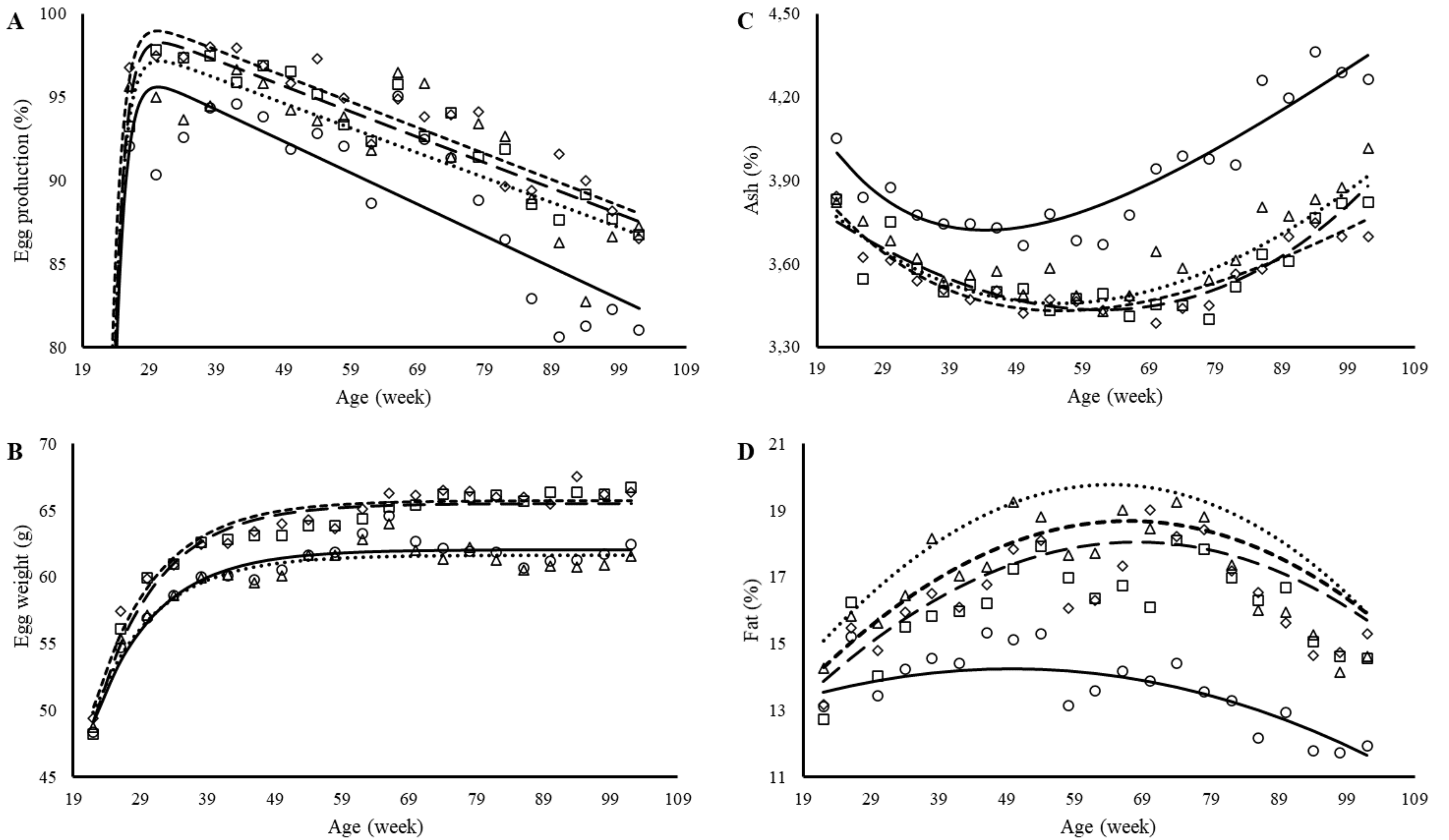

Figure 2. Observed and predicted egg production (A), egg weight (B), body ash (C) and body fat (D) of laying hens from 19 to 102 weeks old in response to four diets program with distinct levels of dietary balanced protein feeds. Treatments: LL (○, —). and HH (◊, - -), reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH (□, – –) and HL (Δ, ···), repletion and depletion of BP in laying period

Supplementary tables

Table 11. Mean feed intake, feed conversion ratio and body weight of laying hens from 19 to 102 weeks old subjected to four feeding programs varying balanced protein levels

Age,	Feed intake, g/bird/day						Feed conversion ratio, g/g						Body weight, g/hen					
Weeks[1]	LL[2]	LH	HH	HL	SEM[3]	P-value[4]	LL	LH	HH	HL	SEM	P-value	LL	LH	HH	HL	SEM	P-value
22	75.9	78.0	80.2	83.6	1.09	0.0072	5.47	5.57	3.55	4.08	0.072	<.0001	1,366	1,353	1,471	1,493	30.7	0.1191
26	95.6	96.4	102.1	101.0	1.21	0.0042	1.93	1.85	1.84	1.91	0.026	0.0532	1,384	1,422	1,504	1,499	37.0	0.1959
30	102	103	103	105	1.42	0.7736	2.00	1.76	1.77	1.93	0.026	<.0001	1,374	1,433	1,533	1,510	39.8	0.0412
34	105	107	109	108	1.47	0.4976	1.95	1.81	1.84	1.97	0.029	0.0002	1,412	1,488	1,564	1,539	38.4	0.0493
38	110	110	110	112	1.38	0.8584	1.95	1.80	1.76	1.98	0.025	<.0001	1,449	1,526	1,616	1,614	37.2	0.0076
42	106	108	110	108	1.20	0.4911	1.87	1.79	1.79	1.86	0.023	0.0341	1,466	1,550	1,641	1,621	39.1	0.0099
46	109	109	108	110	1.16	0.8096	1.94	1.78	1.76	1.93	0.016	<.0001	1,485	1,577	1,657	1,639	39.7	0.0126
50	110	110	110	110	1.57	0.9864	1.97	1.81	1.80	1.95	0.022	<.0001	1,480	1,592	1,681	1,630	44.3	0.0039
54	110	111	110	110	1.57	0.8905	1.92	1.83	1.80	1.94	0.022	<.0001	1,472	1,604	1,689	1,641	42.0	0.0016
58	110	109	110	111	1.42	0.6231	1.93	1.81	1.81	1.93	0.020	<.0001	1,470	1,625	1,695	1,679	40.2	0.0003
62	113	111	113	112	1.20	0.8242	2.01	1.87	1.89	1.96	0.038	0.0331	1,445	1,622	1,679	1,681	42.9	0.0011
66	115	113	115	115	1.36	0.4333	1.87	1.82	1.83	1.89	0.020	0.0425	1,479	1,632	1,724	1,703	50.0	0.0003
70	115	113	115	114	1.35	0.8360	1.96	1.87	1.85	1.92	0.022	0.0017	1,454	1,631	1,703	1,677	46.5	0.0002
74	113	114	115	113	1.84	0.7147	1.99	1.84	1.84	2.01	0.030	<.0001	1,454	1,669	1,743	1,694	47.2	<.0001
78	109	108	111	112	1.54	0.1796	1.98	1.80	1.77	1.97	0.025	<.0001	1,441	1,655	1,740	1,679	40.9	<.0001
82	103	109	103	106	1.24	0.1401	1.94	1.80	1.76	1.87	0.038	0.0063	1,397	1,654	1,696	1,652	38.7	<.0001
86	106	107	110	105	1.83	0.2565	2.14	1.85	1.87	1.99	0.035	<.0001	1,373	1,649	1,733	1,621	46.3	<.0001
90	108	109	109	109	2.18	0.9971	2.17	1.87	1.79	2.08	0.025	<.0001	1,357	1,630	1,714	1,632	36.9	<.0001
94	109	106	107	109	2.44	0.5900	2.20	1.83	1.79	2.17	0.035	<.0001	1,374	1,589	1,672	1,591	42.8	0.0002
98	114	112	111	112	2.02	0.6480	2.22	1.93	1.90	2.14	0.034	<.0001	1,398	1,565	1,675	1,619	37.3	0.0013
102	111	113	110	113	2.26	0.7231	2.20	1.95	1.98	2.11	0.035	<.0001	1,341	1,621	1,689	1,502	46.8	0.0023

[1]Every four weeks from 19-week-old.

[2]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.

[3]SEM: Stander error of the mean.

[4] $^*P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^\dagger P \leq 0.10$, and n.s. $P > 0.10$

Table 2. Mean egg production, egg weight and egg mass of laying hens from 19 to 102 weeks old subjected to four feeding programs varying balanced protein levels

Age,	Egg production, %						Egg Weight, g						Egg mass, g					
weeks[1]	LL[2]	LH	HH	HL	SEM[3]	P-value[4]	LL	LH	HH	HL	SEM	P-value	LL	LH	HH	HL	SEM	P-value
22	27.0	29.4	45.8	37.3	2.071	<.0001	48.4	48.2	49.4	48.7	0.558	0.7070	13.1	15.0	20.9	18.1	1.101	<.0001
26	92.1	93.2	96.8	95.6	0.970	0.0637	54.7	56.1	57.4	55.4	0.451	0.0433	49.7	52.3	55.6	52.9	0.750	0.0013
30	90.4	97.8	97.5	95.0	0.959	0.0004	56.9	59.9	59.8	57.2	0.561	0.0034	51.4	58.6	58.4	54.3	0.859	<.0001
34	92.6	97.3	97.4	93.7	1.078	0.0228	58.6	61.0	60.9	58.6	0.566	0.0098	54.3	59.4	60.4	54.9	0.900	<.0001
38	94.4	97.5	98.0	94.5	0.721	0.1218	60.0	62.6	62.4	59.9	0.552	0.0013	56.7	61.0	61.9	56.6	0.828	0.0001
42	94.6	95.9	97.9	96.7	0.774	0.3828	60.1	62.8	62.6	60.2	0.499	0.0037	56.9	60.3	61.3	58.1	0.662	0.0124
46	93.8	96.9	96.9	95.8	0.708	0.3657	59.8	63.1	63.4	59.6	0.610	<.0001	56.1	61.1	61.4	57.1	0.691	0.0002
50	91.9	96.5	95.8	94.2	0.918	0.1033	60.5	63.1	64.0	60.1	0.642	<.0001	55.6	60.9	60.6	56.6	0.903	0.0002
54	92.8	95.2	97.3	93.6	0.961	0.1638	61.6	63.9	64.3	61.6	0.639	0.0025	57.2	60.8	61.6	56.8	1.024	0.0011
58	92.1	93.3	94.9	93.8	1.068	0.4855	61.9	63.8	63.6	61.6	0.642	0.0067	56.3	59.6	60.8	57.8	0.988	0.0185
62	88.7	92.3	92.2	91.8	1.783	0.1998	63.3	64.4	65.1	62.8	0.642	0.0603	56.1	59.4	60.5	57.7	1.188	0.0346
66	95.1	95.8	94.9	96.5	0.739	0.9617	64.6	65.3	66.3	64.0	0.637	0.0660	61.4	61.9	62.9	60.8	0.922	0.5594
70	92.5	92.7	93.8	95.8	1.377	0.5037	62.7	65.4	66.1	62.0	0.637	<.0001	59.1	60.5	62.0	58.4	0.846	0.0567
74	91.4	94.1	93.9	91.4	1.497	0.3197	62.1	66.2	66.5	61.3	0.661	<.0001	56.8	62.2	62.4	56.0	1.073	<.0001
78	88.8	91.4	94.1	93.4	1.637	0.0431	62.0	66.0	66.4	62.2	0.596	<.0001	55.0	60.3	62.5	57.1	1.095	<.0001
82	86.4	91.9	89.6	92.6	1.579	0.0083	61.8	66.1	66.0	61.3	0.698	<.0001	53.5	60.7	59.1	56.7	1.033	<.0001
86	82.9	88.6	89.4	89.0	1.697	0.0016	60.7	65.7	66.0	60.6	0.717	<.0001	49.5	58.1	59.2	53.8	1.089	<.0001
90	80.6	87.7	91.6	86.3	1.802	<.0001	61.2	66.4	65.4	60.8	0.638	<.0001	49.4	58.2	61.5	52.4	1.213	<.0001
94	81.2	89.2	90.0	82.8	1.711	<.0001	61.3	66.3	67.5	60.8	0.692	<.0001	49.8	58.1	60.4	50.3	1.376	<.0001
98	82.3	87.7	88.2	86.6	1.869	0.0515	61.7	66.2	66.2	60.9	0.724	<.0001	51.7	58.0	57.3	52.8	1.232	<.0001
102	81.0	86.8	86.5	87.2	2.061	0.0078	62.4	66.7	66.4	61.5	0.645	<.0001	50.7	57.9	56.1	52.6	1.460	<.0001

[1]Every four weeks from 19-week-old.
[2]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
[3]SEM: Stander error of the mean.
[4] *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001, †P ≤ 0.10, and n.s. P > 0.10

Table 3. Body components of laying hens from 19 to 102 weeks old subjected to four feeding programs varying balanced protein levels

Age,	Ash, %						Fat, %						Protein, %					
weeks[1]	LL[2]	LH	HH	HL	SEM[3]	P-value[4]	LL	LH	HH	HL	SEM	P-value	LL	LH	HH	HL	SEM	P-value
22	4.05	3.83	3.84	3.82	0.061	0.5059	13.1	12.7	13.2	14.3	0.445	0.5405	18.4	18.5	18.4	17.9	0.144	0.4260
26	3.84	3.54	3.62	3.76	0.081	0.1294	15.2	16.2	15.5	15.8	0.863	0.9157	17.3	17.1	17.5	17.3	0.332	0.8762
30	3.87	3.75	3.61	3.68	0.069	0.0722	13.4	14.0	14.8	15.6	0.619	0.1809	18.1	18.0	17.9	17.4	0.242	0.2241
34	3.77	3.58	3.54	3.62	0.068	0.1048	14.2	15.5	16.0	16.5	0.730	0.2571	17.9	17.5	17.5	17.2	0.270	0.3098
38	3.74	3.50	3.51	3.54	0.064	0.0525	14.6	15.8	16.5	18.1	0.805	0.0209	17.8	17.5	17.3	16.6	0.320	0.0196
42	3.74	3.52	3.47	3.56	0.064	0.0237	14.4	16.0	16.1	17.0	0.740	0.0800	17.9	17.5	17.6	17.0	0.280	0.1486
46	3.73	3.50	3.50	3.57	0.068	0.0695	15.3	16.2	16.8	17.3	0.796	0.3177	17.5	17.4	17.3	17.0	0.296	0.5432
50	3.66	3.51	3.42	3.49	0.063	0.0131	15.1	17.2	17.8	19.2	0.723	0.0009	17.6	17.0	16.9	16.3	0.239	0.0106
54	3.78	3.43	3.47	3.59	0.066	0.0019	15.3	17.9	18.1	18.8	0.751	0.0076	17.5	16.7	16.8	16.3	0.260	0.0198
58	3.68	3.48	3.46	3.48	0.057	0.0055	13.2	17.0	16.1	17.6	0.652	<.0001	18.5	17.2	17.7	16.9	0.224	0.0003
62	3.67	3.49	3.43	3.43	0.063	0.0079	13.6	16.3	16.3	17.7	0.808	0.0024	18.3	17.5	17.6	17.0	0.266	0.0055
66	3.78	3.41	3.48	3.49	0.063	0.0004	14.2	16.7	17.3	19.0	0.771	0.0002	18.0	16.9	17.2	16.4	0.307	0.0003
70	3.94	3.45	3.39	3.65	0.076	<.0001	13.9	16.1	19.0	18.5	0.840	<.0001	18.0	17.6	16.8	16.5	0.288	0.0001
74	3.99	3.45	3.44	3.59	0.075	<.0001	14.4	18.1	18.2	19.2	0.815	<.0001	17.7	16.7	16.8	15.9	0.263	0.0006
78	3.98	3.40	3.45	3.54	0.073	<.0001	13.5	17.8	18.4	18.8	0.851	<.0001	18.1	16.9	16.7	16.4	0.295	<.0001
82	3.96	3.52	3.56	3.61	0.084	0.0002	13.3	17.0	17.2	17.4	0.745	<.0001	18.2	17.2	17.1	16.9	0.262	0.0027
86	4.26	3.63	3.58	3.80	0.088	<.0001	12.2	16.3	16.5	16.0	0.656	<.0001	18.5	17.4	17.4	17.3	0.228	0.0066
90	4.20	3.61	3.70	3.77	0.099	<.0001	12.9	16.7	15.6	15.9	0.806	0.0076	18.3	17.2	17.7	17.4	0.276	0.0580
94	4.36	3.77	3.75	3.83	0.081	<.0001	11.8	15.1	14.6	15.3	0.643	0.0016	18.7	17.8	18.1	17.6	0.229	0.0349
98	4.29	3.82	3.70	3.87	0.071	<.0001	11.7	14.6	14.7	14.1	0.533	0.0290	18.8	18.0	18.1	18.1	0.200	0.1530
102	4.26	3.82	3.70	4.02	0.099	0.0020	11.9	14.5	15.3	14.6	0.870	0.0529	18.6	17.9	18.3	17.7	0.296	0.0678

[1]Every four weeks from 19-week-old.
[2]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
[3]SEM: Stander error of the mean.
[4] $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $†P \leq 0.10$, and n.s. $P > 0.10$

Table 4. Egg components of laying hens from 19 to 102 weeks old subjected to four feeding programs varying balanced protein levels

Age,	Yolk, g						Sell, g						Albumen, g					
weeks[1]	LL[2]	LH	HH	HL	SEM[3]	P-value[4]	LL	LH	HH	HL	SEM	P-value	LL	LH	HH	HL	SEM	P-value
22	10.7	10.8	11.2	10.9	0.137	0.5660	5.65	5.69	5.87	5.59	0.067	0.1183	34.9	34.6	35.0	35.0	0.345	0.9653
26	13.2	14.0	14.4	13.5	0.196	0.0085	5.81	6.05	6.19	5.87	0.073	0.0036	36.4	38.6	38.6	36.9	0.420	0.0057
30	14.3	15.1	15.3	14.7	0.218	0.0123	5.92	6.14	6.09	5.84	0.075	0.0487	38.2	39.3	39.2	38.0	0.427	0.1948
34	15.2	15.5	15.7	15.3	0.205	0.4161	6.07	6.14	6.27	5.87	0.088	0.0050	38.5	39.8	39.8	38.6	0.487	0.0907
38	15.9	16.2	16.4	15.4	0.282	0.0608	6.21	6.23	6.33	6.11	0.082	0.2930	38.5	40.0	39.6	38.2	0.524	0.0454
42	16.0	16.7	17.0	15.7	0.239	0.0008	6.08	6.11	6.15	5.95	0.059	0.2600	39.4	40.4	40.5	37.9	0.563	0.0050
46	15.9	16.8	17.0	16.1	0.274	0.0079	6.05	6.19	6.26	6.05	0.073	0.1502	38.2	40.6	40.2	38.0	0.570	0.0002
50	16.3	17.3	17.2	16.2	0.246	0.0010	5.88	6.08	6.10	5.84	0.080	0.0192	38.2	40.8	40.4	38.2	0.544	<.0001
54	16.8	17.0	17.5	17.3	0.239	0.2910	6.08	6.13	6.22	6.14	0.076	0.6143	39.7	40.6	40.8	39.6	0.551	0.1986
58	16.5	17.3	17.3	16.6	0.251	0.0301	6.00	6.16	6.20	6.00	0.076	0.1340	39.6	40.6	40.8	39.0	0.519	0.0673
62	17.3	17.5	17.9	17.5	0.250	0.4225	6.03	6.07	6.19	6.02	0.098	0.4820	41.1	41.3	41.3	41.2	0.546	0.9943
66	17.6	17.8	18.4	17.6	0.247	0.0452	6.16	6.09	6.31	6.18	0.078	0.2532	41.3	41.4	41.6	40.8	0.500	0.7695
70	16.8	17.7	18.4	16.6	0.287	<.0001	5.94	6.16	6.31	5.96	0.066	0.0003	40.3	42.3	41.8	39.4	0.583	0.0017
74	17.0	18.4	18.3	16.8	0.259	<.0001	5.89	6.17	6.12	5.88	0.073	0.0113	39.7	42.2	41.7	39.8	0.535	0.0022
78	16.5	17.8	18.5	16.7	0.240	<.0001	5.75	5.98	6.12	5.78	0.085	0.0128	39.8	41.8	42.9	39.8	0.578	<.0001
82	16.7	18.1	18.3	16.3	0.243	<.0001	5.60	6.04	6.05	5.62	0.072	<.0001	39.5	43.1	41.9	38.9	0.643	<.0001
86	16.8	17.6	18.3	16.1	0.287	<.0001	5.62	5.90	6.05	5.49	0.092	<.0001	39.6	41.4	42.2	38.6	0.564	<.0001
90	16.1	17.9	18.4	16.1	0.292	<.0001	5.67	6.04	6.11	5.56	0.094	<.0001	39.8	42.9	43.0	39.5	0.614	<.0001
94	16.0	18.0	17.9	16.3	0.276	<.0001	5.61	5.90	5.88	5.59	0.100	0.0268	39.7	43.1	42.2	39.2	0.643	<.0001
98	16.1	18.0	18.1	16.2	0.283	<.0001	5.47	5.86	5.91	5.71	0.104	0.0065	40.6	43.8	42.9	39.4	0.680	<.0001
102	17.0	18.1	18.1	16.1	0.298	<.0001	5.63	5.80	5.86	5.45	0.104	0.0358	41.2	42.7	41.9	40.7	0.678	0.2311

[1]Every four weeks from 19-week-old.
[2]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
[3]SEM: Stander error of the mean.
[4] $^{*}P \le 0.05$, $^{**}P \le 0.01$, $^{***}P \le 0.001$, †$P \le 0.10$, and n.s. $P > 0.10$

Table 5. Shell quality of laying hens from 19 to 102 weeks old subjected to four feeding programs varying balanced protein levels

Age,	Shell strength, kgf						Shell thickness, mm					
weeks[1]	LL[2]	LH	HH	HL	SEM[3]	P-value[4]	LL	LH	HH	HL	SEM	P-value
22	5.81	5.77	5.77	5.66	0.077	0.7426	0.448	0.437	0.450	0.439	0.005	0.3163
26	5.59	5.58	5.71	5.59	0.082	0.7589	0.404	0.405	0.414	0.408	0.003	0.2117
30	5.28	5.42	5.38	5.16	0.089	0.2976	0.395	0.399	0.393	0.389	0.004	0.5576
34	5.38	5.18	5.36	4.77	0.097	0.0001	0.404	0.401	0.400	0.388	0.005	0.0980
38	5.33	5.25	5.29	5.22	0.109	0.9017	0.402	0.401	0.406	0.403	0.004	0.8273
42	5.07	5.14	5.13	5.08	0.089	0.9552	0.394	0.386	0.394	0.388	0.003	0.3129
46	5.01	5.03	5.14	5.02	0.103	0.8108	0.398	0.397	0.396	0.399	0.004	0.9572
50	4.65	4.89	5.00	4.63	0.119	0.0289	0.403	0.392	0.400	0.395	0.004	0.2831
54	4.67	4.85	4.73	4.74	0.103	0.6700	0.384	0.384	0.387	0.387	0.005	0.9328
58	4.70	4.77	4.56	4.64	0.115	0.5614	0.385	0.382	0.388	0.387	0.004	0.7553
62	4.46	4.41	4.57	4.29	0.096	0.2716	0.386	0.389	0.390	0.390	0.005	0.9140
66	4.14	4.23	4.34	4.12	0.102	0.4539	0.387	0.371	0.379	0.384	0.003	0.0169
70	3.98	4.12	4.12	4.18	0.104	0.6710	0.368	0.368	0.378	0.374	0.004	0.1156
74	3.79	4.04	3.80	3.88	0.111	0.2943	0.368	0.370	0.370	0.371	0.004	0.9559
78	3.76	3.86	3.88	4.00	0.102	0.6140	0.357	0.359	0.363	0.356	0.004	0.5874
82	3.33	3.64	3.65	3.43	0.101	0.0681	0.353	0.361	0.360	0.352	0.003	0.1104
86	3.30	3.61	3.47	3.39	0.110	0.1733	0.353	0.355	0.357	0.349	0.005	0.6037
90	3.35	3.58	3.62	3.19	0.106	0.0141	0.349	0.356	0.358	0.353	0.004	0.3807
94	3.29	3.36	3.77	3.41	0.115	0.0134	0.353	0.352	0.355	0.356	0.005	0.9442
98	3.24	3.20	3.31	3.41	0.100	0.4905	0.361	0.368	0.370	0.376	0.005	0.1622
102	3.17	3.22	3.28	3.07	0.094	0.5019	0.341	0.345	0.345	0.343	0.004	0.9010

[1]Every four weeks from 19-week-old.
[2]LL and HH, reduction and accretion of using balanced protein (BP) along rearing period and laying period; LH and HL, repletion and depletion of BP in laying period.
[3]SEM: Stander error of the mean.
[4] $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $\dagger P \leq 0.10$, and n.s. $P > 0.10$

CAPÍTULO 4 – Implicações

Visto que os avanços genéticos têm sido direcionados principalmente para o aumento da persistência de produção de ovos, proporcionando a extensão do ciclo de postura. A otimização do desempenho produtivo está atrelada a eficiência alimentar e a nutrição acompanhou o progresso genético. Para o nutricionista, é essencial compreender como o aumento ou redução da proteína balanceada na dieta afeta o desenvolvimento das frangas e o potencial de produção de ovos e a extensão do ciclo produtivo das aves.

Para os produtores de ovos comerciais, existe uma grande preocupação com os custos de produção e a necessidade de otimização dos custos implica em reduzir os níveis nutricionais da dieta no período de recria. A recria é considerado pelos produtores como um período “sem retorno econômico”, neste cenário, é necessário trabalhar com programas de dieta acima das recomendações nutricionais mínimas no período de recria para que não prejudique o desempenho produtivo subsequente. Embora a redução da proteína balanceada na dieta não afete a composição corporal das aves, tal estratégia nutricional pode atrasar o grau de maturidade das frangas modificando a curva de produção das aves o peso inicial dos ovos.

Os manuais de linhagem modernas recomendam o fornecimento de nutrientes dietéticos para expressão do potencial genético. Para os produtores, a redução do nível proteico ao longo do período de postura pode ser uma alternativa para melhorar a rentabilidade produtiva. Contudo, é necessário que as frangas de postura sejam bem desenvolvidas para manter a produção de ovos a longo prazo. Para atingir este objetivo é importante entender como a redução da proteína balanceada da dieta impacta a produção de ovos. De acordo com este trabalho, a redução da proteína balanceada ao longo do período de postura de galinhas submetidas a dietas de alta nutrição proteica durante o período de recria, não afetou a produção de ovos, contudo reduziu o peso e a massa do ovo e com base nesses resultados, o produtor pode tomar sua decisão.

Entender como os níveis de proteína balanceada afeta a dinâmica da composição corporal e dos componentes do ovo ao longo dos ciclos de produção, permite o desenvolvimento de modelos de simulação e para estimar o desempenho produtivo mediante diferentes estratégias nutricionais. Os resultados deste trabalho também permitiram determinar como os níveis de proteína balanceada afeta a composição corporal das galinhas e o reflexo disso na produção de ovos e determinar o efeito dos níveis proteicos nas relações alométricas dos componentes do ovo.