

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

**ESTUDO DE FONTES E EXIGÊNCIA DE
TRIPTOFANO PARA POEDEIRAS SEMIPESADAS**

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

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TRIPTOFANO PARA POEDEIRAS SEMIPESADAS**

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DADOS CURRICULARES DO AUTOR

Stéphane Cristyne de Oliveira Estevão, filha de Baltazar Estevão e Edna de Fátima Oliveira Estevão, nasceu em 10 de março de 1993, em Patos de Minas, Minas Gerais. Em 2018, concluiu o bacharelado em Zootecnia pelo Centro Universitário de Patos de Minas, onde foi bolsista de iniciação científica sob a orientação da professora Mestra Cristiane Quitéria Caldeira e teve a oportunidade de realizar intercâmbio acadêmico em Nebraska, EUA. Em 2020, obteve o título de Mestre em Zootecnia, com ênfase em Produção Animal, pela Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Campus Diamantina, sob a orientação da Professora Doutora Sandra Regina de Freitas, com bolsa da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Atualmente, é Doutoranda no Programa de Pós-Graduação em Ciência Animal da Faculdade de Ciências Agrárias e Veterinárias da Universidade Estadual Paulista "Júlio de Mesquita Filho" (FCAV/UNESP), Campus Jaboticabal, sob a orientação do Professor Doutor Edney Pereira da Silva, durante seu doutorado, foi bolsista da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Aos meus pais, Baltazar e Edna,
fonte de amor incondicional e exemplo de bondade,
com todo meu amor e gratidão.
Dedico.

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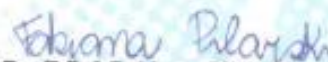
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CERTIFICADO

Certificamos que o projeto de pesquisa intitulado "**Study of tryptophan sources and doses for brown laying hens**", protocolo nº 3340/22, sob a responsabilidade do Prof. Dr. Edney Pereira da Silva, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao Filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) - encontra-se de acordo com os preceitos da lei nº 11.794, de 08 de outubro de 2008, no decreto 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), da FACULDADE DE CIÊNCIAS AGRÁRIAS E VETERINÁRIAS, UNESP - CÂMPUS DE JABOTICABAL-SP, em reunião ordinária de 15 de junho de 2022.

Vigência do Projeto	01/07/2022 a 30/08/2027
Espécie / Linhagem	<i>Gallus Gallus domesticus</i> / Poedeiras comerciais/ Hy-line Brown
Nº de animais	1000
Peso / Idade	1,6 – 1,7 kg / 52 semanas
Sexo	Fêmeas
Origem	Sítio Yaiota – Mirandópolis/SP. 168000-000

Jaboticabal, 15 de junho de 2022.


Profª Drª Fabiana Pilarski
Coordenadora – CEUA

Título: ESTUDO DE FONTES E EXIGÊNCIA DE TRIPTOFANO PARA POEDEIRAS SEMIPESADAS

Resumo

Esta pesquisa foi estruturada de forma abrangente para validar a eficácia técnica e estabelecer as recomendações nutricionais precisas para o uso da biomassa de L-triptofano 60% em dietas de poedeiras comerciais. O estudo foi dividido em duas etapas experimentais, utilizando fontes de referência para consolidar o potencial deste ingrediente na avicultura moderna. Na primeira fase, o objetivo foi avaliar a bioequivalência e determinar o valor de biodisponibilidade relativa (RBV) da biomassa em comparação com a fonte de referência, o L-triptofano 98%. Para isso, foram realizados dois ensaios simultâneos com um total de 216 aves. O primeiro ensaio utilizou o método de comparação direta, essencial para verificar se a biomassa se comporta de forma idêntica à fonte purificada sob condições controladas. O segundo ensaio aplicou o método de slope ratio (razão de inclinação), uma técnica estatística avançada para calcular o RBV. As variáveis analisadas nesta etapa foram: O desempenho produtivo, consumo de ração, ingestão de triptofano, produção de ovos, peso médio do ovo, massa de ovos, conversão alimentar e eficiência alimentar. Sobre a qualidade dos ovos foi medido o peso da gema, da casca e do albúmen, altura do albúmen, espessura da casca e unidade Haugh. Além da composição corporal, que se avaliou a porcentagem de proteína, lipídios, água e cinzas no corpo das aves, visando entender o particionamento de nutrientes. Os resultados foram tabulados e realizado a análise de variância (ANOVA). No método de slope ratio, apenas a produção de ovos e a ingestão diária de triptofano apresentaram efeitos que permitiram o ajuste de equações lineares. Ao aplicar o cálculo de slope ratio nessas equações, determinou-se um RBV de 100%, provando que a biomassa de L-triptofano 60% é bioequivalente e possui a mesma eficiência metabólica que a fonte purificada. A segunda fase da pesquisa focou na reavaliação das exigências de triptofano para poedeiras marrons em fase final de produção (aves idosas), utilizando especificamente a biomassa. O intuito foi atualizar as recomendações nutricionais e investigar se o produto apresenta algum efeito adjuvante na dieta. Este experimento utilizou 264 galinhas, distribuídas em oito tratamentos experimentais. As análises detectaram que os níveis de triptofano influenciaram significativamente ($P \leq$

0,05) apenas as variáveis de desempenho, sem alterar a qualidade dos ovos. Para identificar a ingestão ideal, foram ajustados modelos de linear-plateau (LP) e quadratic-plateau (QP). Os pontos de intercepto ideais calculados foram: 186,2 mg/ave/dia para produção de ovos; 174,7 mg/ave/dia para massa de ovos; 182,7 mg/ave/dia para conversão alimentar e 182,5 mg/ave/dia para eficiência alimentar. Ao integrar todos os dados, especialmente a relação entre a ingestão do aminoácido e sua deposição final no ovo, a pesquisa estimou que a ingestão ótima de triptofano é de 187 mg/ave/dia. Este nível assegura a máxima eficiência de utilização do aminoácido pelas galinhas, confirmando que a biomassa de L-triptofano 60% é uma alternativa segura, bioequivalente e altamente eficaz para a formulação de dietas avícolas.

Palavras-chave: Aminoácidos, bioequivalência, exigência nutricional, poedeiras.

Title: STUDY OF TRYPTOPHAN SOURCES AND REQUIREMENTS
FOR SEMI-HEAVY LAYING HENS

Abstract

This research was comprehensively structured to validate the technical efficacy and establish precise nutritional recommendations for the use of 60% L-tryptophan biomass in commercial laying hen diets. The study was divided into two experimental phases, using reference sources to consolidate the potential of this ingredient in modern poultry farming. In the first phase, the objective was to evaluate the bioequivalence and determine the relative bioavailability (RBV) value of the biomass compared to the reference source, 98% L-tryptophan. For this, two simultaneous trials were conducted with a total of 216 birds. The first trial used the direct comparison method, essential to verify if the biomass behaves identically to the purified source under controlled conditions. The second trial applied the slope ratio method, an advanced statistical technique to calculate the RBV. The variables analyzed in this stage were: productive performance, feed consumption, tryptophan intake, egg production, average egg weight, egg mass, feed conversion, and feed efficiency. Egg quality was measured by measuring yolk weight, shell weight, albumen weight, albumen height, shell thickness, and Haugh unit. Body composition was also assessed, evaluating the percentage of protein, lipids, water, and ash in the birds' bodies to understand nutrient partitioning. The results were tabulated and an analysis of variance (ANOVA) was performed. In the slope ratio method, only egg production and daily tryptophan intake showed effects that allowed the adjustment of linear equations. Applying the slope ratio calculation to these equations, a RBV of 100% was determined, proving that the 60% L-tryptophan biomass is bioequivalent and has the same metabolic efficiency as the purified source. The second phase of the research focused on reassessing the tryptophan requirements for brown laying hens in the final production phase (older birds), specifically using biomass. The aim was to update nutritional recommendations and investigate whether the product has any adjuvant effect on the diet. This experiment used 264 hens, distributed into eight experimental treatments. The analyses detected that tryptophan levels significantly influenced ($P \leq 0.05$) only performance variables, without altering egg quality. To identify the ideal intake, linear-plateau (LP) and quadratic-

plateau (QP) models were adjusted. The calculated ideal intercept points were: 186.2 mg/bird/day for egg production; 174.7 mg/bird/day for egg mass; 182.7 mg/bird/day for feed conversion; and 182.5 mg/bird/day for feed efficiency. By integrating all the data, especially the relationship between amino acid intake and its final deposition in the egg, the research estimated that the optimal tryptophan intake is 187 mg/bird/day. This level ensures maximum efficiency in the use of the amino acid by hens, confirming that L-tryptophan 60% biomass is a safe, bioequivalent, and highly effective alternative for formulating poultry diets.

Keywords: Amino acids, bioequivalence, nutritional requirement, laying hens.

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CAPÍTULO 1. Considerações Gerais

1.1 Introdução

O triptofano (TRP) pertence à classe dos aminoácidos aromáticos e suas funções primárias estão relacionadas às sínteses de proteína, niacina e serotonina (TACKMAN et al., 1990). Ele é considerado o terceiro aminoácido limitante para aves alimentadas com dietas a base de milho e farelo de soja, sucedido pela metionina e lisina (PEGANOVA et al., 2003). Embora o triptofano tenha sido descoberto em 1901 por Hopkins & Cole, sua estrutura só foi esclarecida posteriormente por Ellinger e Flamand, (1907).

Este aminoácido desempenha um papel crucial no ganho de peso, consumo de ração e taxa de conversão alimentar, além de influenciar a condição dos anticorpos (MOZHDEH et al., 2010). Ele também está intimamente relacionado à performance produtiva e atua como um regulador da saúde óssea. A serotonina é um neurotransmissor vital que melhora a adaptabilidade ambiental e alivia o estresse oxidativo (MARTIN et al., 2000). É também uma importante molécula sinalizadora da mucosa produzida pelas células enterocromafins no intestino e está relacionada a numerosos processos fisiopatológicos (COATES et al., 2004).

Além de ser parcialmente incorporado à proteína, o triptofano é metabolizado por vias que produzem compostos biologicamente importantes. Existem duas vias metabólicas principais deste aminoácido (Comai et al., 1997): a via da quinurenina, que dá origem ao ácido nicotínico, precursor das coenzimas nicotinamida adenina dinucleotídeo; e a via da serotonina, que leva à formação do neurotransmissor serotonina e da melatonina, um hormônio e um neuromodulador.

Considerando que os aminoácidos estão entre os nutrientes mais importantes para performance produtiva das poedeiras e, uma vez que as exigências em determinados aminoácidos têm relação direta com a produção de ovos, conversão alimentar e eficiência na utilização de nitrogênio, é relevante a determinação das exigências em aminoácidos para poedeiras (JANSMAN, 2002). Além da importância da determinação da biodisponibilidade

e das exigências de manutenção das fontes de aminoácidos utilizadas nas rações de poedeiras, é igualmente relevante identificar a necessidade específica de cada aminoácido, garantindo assim o máximo desempenho das aves.

2. Revisão de Literatura

2.1. Caracterização do Triptofano

Os aminoácidos são substâncias orgânicas que apresentam em sua molécula um grupo amino e um grupo carboxila e são os componentes das proteínas (LE FLOC'H et al., 2011). Segundo Marzzoco e Torres (2015), os aminoácidos possuem uma fórmula básica comum, na qual os grupos amino e carboxila estão ligados ao carbono α , ao qual também se liga um átomo de hidrogênio e um grupo lateral ou grupo R. São ainda classificados de acordo com a polaridade do grupo R, em duas grandes categorias: apolares (grupo R hidrofóbico) e polares (grupo R hidrofílico).

A síntese de aminoácidos depende de diversos fatores, entre os quais: a disponibilidade de substrato, a espécie, o estágio de desenvolvimento, o estado fisiológico, a microbiota no lúmen do trato gastrointestinal e os fatores ambientais. Este fato, por sua vez, ilustra a dinâmica das exigências nutricionais pelos aminoácidos e a complexidade de classificação dos mesmos como: essenciais, não essenciais ou condicionalmente essenciais (WU et al., 2014).

O conjunto de aminoácidos que serão utilizados para a síntese proteica origina-se das proteínas endógenas resultantes da hidrólise intracelular e das proteínas exógenas obtidas pela digestão das proteínas oriundas da dieta (MARZZOCO & TORRES, 2007). Nas aves, a digestão se inicia no proventrículo, local onde ocorre a secreção de pepsinogênio, que é ativado pelo ácido clorídrico, formando a pepsina (SILVA et al., 2014). Posteriormente, o produto da hidrólise é direcionado ao intestino delgado e, no lúmen, é hidrolisado por proteases pancreáticas (tripsina, quimiotripsina, elastase, carboxipeptidases A e B) e intestinais (aminopeptidases e carboxipeptidases), que fracionam as proteínas em peptídeos menores e quantidades consideráveis de aminoácidos livres (WU, 2013). Os peptídeos compostos por mais de três resíduos de aminoácidos são hidrolisados extracelularmente por

peptidases localizadas principalmente na borda em escova dos enterócitos e, em menor grau, no lúmen intestinal, para formar dipéptidos, tripéptidos e aminoácidos livres (SILVA et al., 2014).

O triptofano quando é fornecido em excesso em uma dieta e não é utilizado para a síntese proteica ele pode ser utilizado como um terapêutico (LIU et al., 2013). Para poedeiras, os efeitos benéficos da suplementação do triptofano têm sido observados na melhoria da eficiência alimentar, principalmente através do processo digestivo e também absoritivo uma vez que, este aminoácido promove equilíbrio da microbiota intestinal por aumentar a concentração de bactérias benéficas como, por exemplo, lactobacilos, bifidobactérias e enterobactérias (KHANIPOUR et al., 2019; KATTAK & HELMBRECHT, 2019; BELLO et al., 2018).

Além disso, é considerado um aminoácido funcional, junto à arginina, cisteína, leucina, metionina, entre outros (WU et al., 2014). Dos vinte aminoácidos que compõem as proteínas, ele é um dos encontrados em menor proporção nas proteínas e no plasma, pois está envolvido em diversas funções fisiológicas (LE FLOC'H et al., 2011).

A sua concentração plasmática é determinada pelo balanço entre a ingestão dietética e sua remoção do plasma pela síntese proteica (SEZINI et al., 2014). Quando hidrolisado pela ação da enzima triptofano hidroxilase (TPH) em 5-hidroxitriptofano (5-HTP), que é rapidamente metabolizado a 5-HT, também conhecida como serotonina. A serotonina produzida é armazenada em vesículas sinápticas, nos neurônios serotoninérgicos, até a sua utilização e/ou metabolização em ácido 5-hidroxiindoleacético (5-HIAA) (GIBSON et al., 2014)

A disponibilidade de triptofano no cérebro para a conversão em 5-HT não depende exclusivamente de sua concentração plasmática, mas também da razão entre o triptofano plasmático e outros cinco aminoácidos neutros de cadeia longa (LNAAs): L-tirosina, L-fenilalanina, L-leucina, L-isoleucina e L-valina. Dessa forma, apenas uma fração limitada do triptofano consegue transpor a barreira hematoencefálica, resultando em uma disponibilidade reduzida em relação aos demais aminoácidos e, conseqüentemente, em uma menor síntese de serotonina (PÓVOA et al., 2005). A associação com carboidratos, nesse contexto, promove o aumento da captação de triptofano

pelo cérebro ao estimular sua passagem pela barreira hematoencefálica, favorecendo a razão TRP/LNAAs (HUDSON et al., 2007).

Vitaminas do complexo B (B3, B6, B12, B9) e os minerais magnésio e cálcio atuam como cofatores na conversão do triptofano para 5-HT de forma satisfatória (OKIGAMI & MONÇÃO, 2011). A vitamina C também será requerida nesse processo, inibindo a conversão do triptofano no trato gastrointestinal (OLSZEWER, 2008).

2.2. Metabolismo do Triptofano

Em 1901, o triptofano foi relatado pela primeira vez por Frederick Hopkins, que descobriu o triptofano através da hidrólise da caseína. O triptofano pode ser encontrado em alguns grãos como fontes naturais, tais como nozes, castanha de caju, amendoim, gergelim, amêndoas, sementes de girassol, abóbora e soja, com altas concentrações (STRASSER et al., 2016). Ele é facilmente absorvido pelos capilares da parede intestinal. A maior parte dele, cerca de 80-90%, é transportada no sangue pela albumina, diferentemente de outros aminoácidos (PARDRIDGE, 1979). Assim, apenas 10-20% do triptofano está presente como forma livre no plasma. Alguns ácidos graxos não esterificados e alguns medicamentos podem modificar sua ligação à albumina (LE FLOC'H et al., 2011).

Como se sabe, a produção de triptofano segue três vias metabólicas (Figura 1), que são: via de síntese proteica (1); via da serotonina e da melatonina (2) e, via da quinurenina (3). Na via da síntese proteica, assim como ocorre com outros aminoácidos, o triptofano está envolvido na produção de proteínas nos corpos dos animais, principalmente através do fígado. A via da serotonina e da melatonina, o triptofano é metabolizado para produzir neurotransmissores essenciais e hormônios reguladores. A rota da quinurenina é a mais significativa das três vias de metabolismo do triptofano. Ela sintetiza a molécula de indol, que é essencial na formação de metabólitos que possuem atividade fisiológica e estão associados à defesa do organismo (NATHALIE & SEVE 2007).

A via serotoninérgica, quando ativada em plaquetas e neurônios, converte o triptofano no neurotransmissor 5-HT. Alguns tecidos são capazes de utilizar 5-HT para síntese do hormônio melatonina numa rota intermediada por

N-acetilserotonina. Na via das quinureninas, o triptofano é oxidado a N-formil-quinurenina (NFK) que subsequentemente é transformado em L-quinurenina (KYN). A partir deste último, uma série de compostos com potencial atividade biológica, especialmente neurotóxica, é formada como, por exemplo: 3-hidroxi-quinurenina e o ácido quinolínico (STONE, 2001; WIDNER et al., 2000).

Já a da quinurenina é a principal via metabólica, através da qual 95% do triptofano da dieta é processado e excretado (DAVIS & LIU 2015). A enzima triptofano hidroxilase que limita a taxa de síntese de serotonina a partir do triptofano, tem atividade parcial de sua capacidade, de tal modo que o aumento da sua disponibilidade pode dobrar a taxa de síntese de serotonina (CARLSON, 1977; HEINE et al., 1995; SAINIO et al., 1996). O transporte e a disposição do triptofano parecem ser fatores limitantes para a síntese de serotonina pelo cérebro (PARDRIDGE, 1998; LE FLOC'H & SÈVE, 2007). A contribuição de triptofano ao metabolismo energético é dupla, por um lado, é cetogênica, ou seja, ela forma acetil coenzima A, e, por outro, é glucogênico, pois produz alanina (GÓMEZ & LLORCA, 2000).

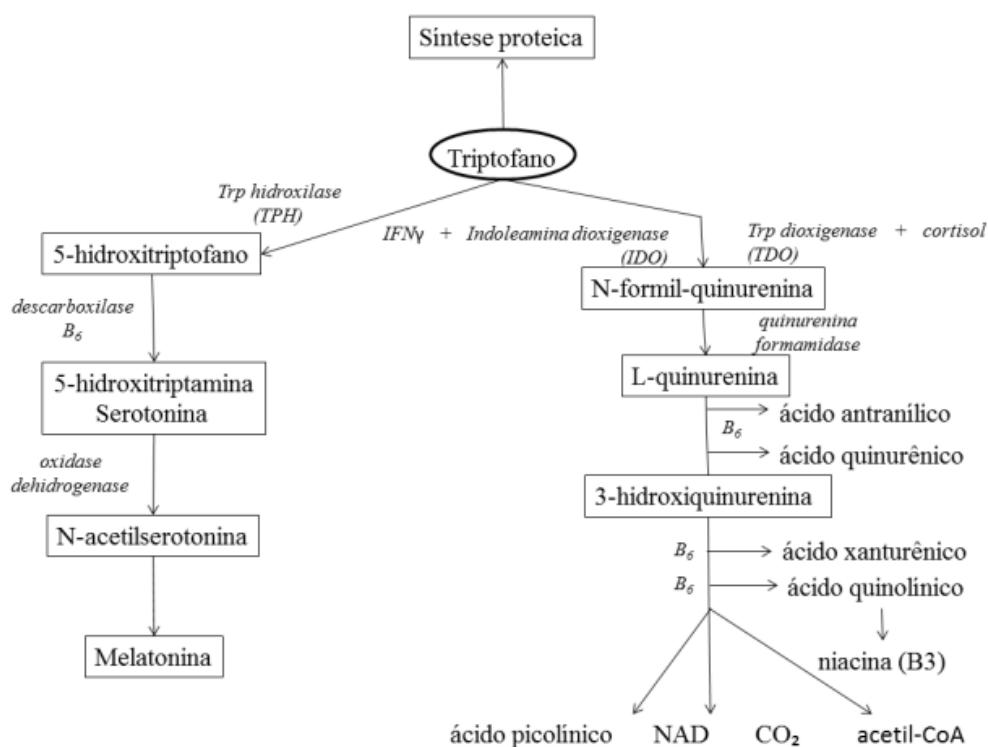


Figura 1. Metabolismo do triptofano (Le Floc'h et al., 2011; Kuribayashi, 2011).

Em outra rota metabólica da 5-HT, numa via alternativa, produz-se o hormônio melatonina sintetizado pela glândula pineal. A síntese da melatonina inicia-se com a serotonina, que é acetilada a N-acetilserotonina, e seguidamente orto-metilada, resultando na melatonina, conforme demonstrado na Figura 2. As enzimas responsáveis por estas duas fases são respectivamente: arilalquilamina N-acetiltransferase e hidroxí-indol-O-metiltransferase (RIBELAYGA et al., 2000).

A regulação da atividade da glândula pineal se dá através da via do trato retino-hipotalâmico, tornando a melatonina como princípio fundamental para a adaptação do indivíduo e da espécie às flutuações temporais cíclicas do meio ambiente (SOARES et al., 2003; MACCHI & BRUCE, 2004). Níveis elevados de 5-HT são detectados em mamíferos durante o dia, decaindo durante a fase noturna. Concomitantemente, ocorre o aumento da melatonina, pela maior conversão da 5-HT (CLAUSTRAT et al., 2005).

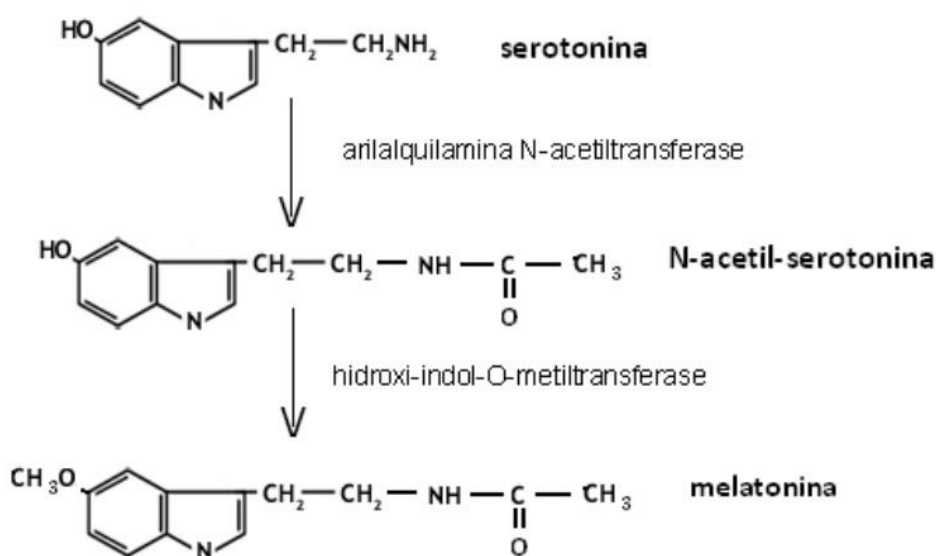


Figura 2. Via de metabolização da 5-HT em melatonina (Pinhati, 2011).

2.3. Partição dos nutrientes: Manutenção

A partição eficaz de nutrientes desempenha um papel vital na produção avícola e afeta diretamente a saúde, o desempenho e a eficiência das aves. No contexto da produção avícola, o termo “manutenção” refere-se ao equilíbrio entre a ingestão e as perdas de nutrientes que garante que as aves mantenham as

suas funções fisiológicas básicas e, quando aplicável, mantenham a produção de ovos ou o ganho de peso conforme necessário (Silva, 2012).

Diversos fatores podem influenciar a eficiência na utilização de aminoácidos em galinhas. Entre eles, destacam-se a saúde, o estado fisiológico das aves e fatores ambientais, como o estresse e a presença de doenças, que podem comprometer a capacidade de metabolizar aminoácidos de forma eficiente (DÖRR, 2019). De acordo com Leveille e Fisher (1959) a manutenção ocorre em aves, especialmente em poedeiras, quando elas conseguem manter a produção de ovos e sua sobrevivência, com a ingestão adequada de nutrientes para equilibrar o processo de absorção e excreção.

Durante a manutenção, ocorrem perdas significativas de aminoácidos através de várias vias metabólicas. Essas incluem a perda de aminoácidos via pele e penas, as perdas endógenas de aminoácidos do intestino, a excreção de aminoácidos livres na urina e a perda de proteína corporal (Moughan, 2003; BONATO et al., 2011). Para manter o equilíbrio de nitrogênio e garantir uma eficiente utilização de nutrientes, os aminoácidos devem ser fornecidos na mesma proporção em que são perdidos pelo metabolismo, secreção e excreção (SAKOMURA E ROSTAGNO, 2007). As perdas endógenas de aminoácidos, especialmente no cólon e no íleo, desempenham um papel crucial, afetando a verdadeira digestibilidade dos aminoácidos em aves (VELMURUGU, 2021; CARLENE et al., 2018). Essas perdas têm impacto direto na utilização de proteínas e nas necessidades dietéticas das aves, sendo influenciadas por componentes específicos da dieta, como fibras e antinutrientes (SOOMRO et al., 2017; SIRIWAN et al., 2012; CERRATE et al., 2012).

A qualidade e a composição da dieta são fatores-chave na distribuição de nutrientes para as aves. Estudos realizados por Alves (2018) e Paiva (2014) destacaram a importância da presença e proporções adequadas de aminoácidos nas dietas das aves para garantir um crescimento saudável e uma produção eficiente de ovos. Além disso, as interações entre os diferentes nutrientes da dieta também podem afetar a sua absorção e utilização (ALVES, 2018).

O processamento e armazenamento de rações também são fatores relevantes que afetam a disponibilidade e a qualidade dos nutrientes para as

aves. Pesquisa realizada por Tinoco (2012) mostram que o processamento ou armazenamento inadequado de rações pode levar a perdas significativas de nutrientes, comprometendo assim a eficiência da distribuição de nutrientes.

Matematicamente, a relação entre o aminoácido depositado e sua ingestão é equivalente à eficiência, o que significa que, conhecendo-se o depositado ou ganho de aminoácido, ao dividir pelo índice de utilização, é possível prever a quantidade necessária de ingestão de aminoácido ($\text{depositado/eficiência} = \text{ingestão}$). Dessa forma, tanto os modelos empíricos quanto os mecânicos são capazes de prever a demanda de aminoácidos (SILVA et al., 2012).

Resumidamente, a partição eficaz de nutrientes, particularmente focada na manutenção, é essencial para assegurar a saúde, o desempenho e a produtividade ótima das aves. É fundamental compreender plenamente os elementos que afetam essa distribuição para aprimorar a alimentação e o cuidado das aves em sistemas de criação avícola.

2.4. Qualidade do ovo

O triptofano dietético aumenta a concentração do fator de crescimento semelhante à insulina I (Pan et al., 2013) e diminui os níveis de corticosterona (Bello et al., 2018) em aves. O fator de crescimento semelhante à insulina I regula positivamente a expressão do gene da ovoalbumina (Fu et al., 2001; Kida et al., 1995), enquanto a corticosterona regula negativamente (Kim & Choi, 2014), o que pode refletir positivamente a produção de ovoalbumina (o principal componente da clara do ovo) e indicar como o triptofano pode afetar a composição da clara do ovo. No entanto, o nível de triptofano relacionado à regulação da secreção de ovoalbumina não é conhecido no momento.

Dentre os diversos índices de qualidade do ovo, a qualidade da casca (espessura, densidade e peso da casca) e a composição do ovo (proporção entre gema e albúmen) são afetadas pela concentração de triptofano na dieta. A dieta deve conter pelo menos 0,18% de triptofano para aumentar os níveis totais de proteína e cálcio no sangue, indicando assim que o metabolismo e a utilização de proteína e cálcio são melhorados, conforme refletido pela proporção de albúmen e qualidade da casca do ovo (KHATTAK & HELMBRECHT, 2019; WEN et al., 2019).

A melatonina, um metabólito do triptofano, ela demonstrou melhorar a captação de Ca^{2+} em estudos realizados em frangos sob estresse (CARPENTIERI et al., 2014). Além disso, foi observado que ela aumenta a capacidade antioxidante intestinal, a secreção de mucina, a resistência das células do epitélio intestinal, a secreção de enzimas digestivas, os transportadores de carboidratos e aminoácidos, bem como promove o crescimento de bactérias intestinais benéficas (AKBARIAN et al., 2014; LI et al., 2017; LIU et al., 2018). A melatonina também foi associada ao aumento da absorção de nutrientes como o Ca^{2+} e contribuiu para elevar os níveis de cálcio no sangue, melhorando, assim, a qualidade da casca do ovo. Evidências sugerem que a serotonina influencia o transporte ativo de eletrólitos intestinais, aumentando a permeabilidade da membrana plasmática intestinal ao cálcio e, conseqüentemente, elevando o cálcio intracelular (DONOWITZ et al., 1980). De acordo com Khattak & Helmbrecht (2019), pode-se levantar a hipótese de que a suplementação de triptofano na dieta das galinhas facilita positivamente a absorção e disponibilidade de cálcio, resultando em uma melhora na qualidade da casca dos ovos.

Segundo Russel & Harms (1999) em seus estudos sugeriram que níveis abaixo de 0,13% triptofano causam redução na produção de ovos e peso corporal de galinhas poedeiras. Além disso, atenção especial deve ser dada às dietas formuladas com ingredientes que apresentem alta concentração de aminoácidos neutros de cadeia longa, tais como, isoleucina, valina e leucina, fenilalanina, tirosina, uma vez que estes aminoácidos influenciam no requerimento de triptofano (PEGANOVA & EDER, 2003). O aumento na produção de ovos através da suplementação de L-triptofano na dieta de poedeiras pode ser explicado pelo efeito do triptofano em melhorar a liberação de gonadotropina, bem como melhorar a disponibilidade de proteína (DONG et al., 2012).

Em outros estudos os níveis de 0,10% a 0,31% do triptofano digestível em dietas à base de milho e trigo para poedeiras marrons durante o pico de produção de ovos foram observados e, notou-se que a dieta com 0,31% teve um consumo de ração maior de 2,7% em comparação a dieta formulada com 0,10% de triptofano digestível. Além disso, esses autores analisaram o efeito do triptofano na qualidade do ovo, onde a análise de regressão mostrou que

um nível de triptofano digestível na dieta de 0,22% promoveu melhor qualidade da casca do ovo, tanto em termos de espessura quanto de densidade, correspondendo à relação ácido triptofano: lisina digestível 27,5% (KATTHAK & HELMBRECHT, 2019).

2.5. Papel do triptofano na resposta ao estresse e adaptação das aves

Estudos recentes investigaram o papel do triptofano na resposta ao estresse e na adaptação em aves, especialmente em ambientes de produção intensiva. A disponibilidade dietética de triptofano está associada à regulação de hormônios do estresse, como corticosterona e cortisol, bem como à síntese de proteínas de choque térmico e serotonina em aves em condições fisiológicas (BELLO, et al., 2018; PAN et al., 2013; WEN et al., 2019).

Esses hormônios determinam o status de estresse nas aves (Archer, 2017; Kang et al., 2019; Shi et al., 2019) e, portanto, pode representar um importante aditivo dietético para mitigar as consequências do estresse nas aves. A serotonina é conhecida como um neurotransmissor cerebral central (O'mahony et al., 2015), que regula o comportamento agressivo e a resposta ao estresse (MARKUS et al., 2000; PUGLISI-ALLEGRA & ANDOLINA, 2015).

A suplementação de triptofano na dieta também pode reduzir a agressão e diminuir o estresse em muitas espécies, incluindo galinhas (HIERDEN et al., 2004). Além disso, a deficiência na dieta pode afetar todos os tipos de funções fisiológicas (BIRKL et al., 2017). Por outro lado, o aumento na suplementação pode diminuir o estresse oxidativo e ajudar as galinhas a lidar com a condição de estresse (WANG et al., 2015).

Comportamentos avícolas, como, humor, processo metabólico, sono e crescimento são afetados pela serotonina central (HAAS & EIJK, 2018). Estudos recentes demonstram que a quantidade de triptofano na dieta afeta a resposta ao estresse e o comportamento de bicar das aves por meio da via indolica (CRUMEYROLLE-ARIAS et al., 2014; YILDIRIM et al., 2020). Além disso, Bai et al. (2017) evidenciaram o papel do triptofano na modulação de funções biológicas e na redução do estresse, enquanto os parâmetros de avaliação do estresse oxidativo incluem sistemas enzimáticos e não

enzimáticos, como catalase, glutathione redutase e peroxidase, bem como o estado antioxidante total.

2.6. Biodisponibilidade

Digestibilidade e biodisponibilidade de alimentos são termos que são frequentemente confundidos, a digestibilidade de alimentos refere-se à porcentagem de nutrientes absorvidos dos alimentos ingeridos, determinada pela diferença entre a quantidade consumida e a quantidade excretada nas fezes (SANTANA, 2013). A biodisponibilidade refere-se à quantidade dos minerais presentes na dieta que são absorvidos e aproveitados para as funções orgânicas normais (BERTECHINI, 2012). Ou seja, a biodisponibilidade envolve os processos de digestão, absorção, metabolismo e utilização do nutriente (SAKOMURA & ROSTAGNO, 2007).

O termo biodisponibilidade, originalmente proposto para a área da farmacologia, indica a proporção de um nutriente absorvida e utilizada pelo organismo. Existem vários fatores intrínsecos e extrínsecos que afetam o melhor ou pior aproveitamento dos nutrientes. Dentre esses fatores intrínsecos podemos destacar: o tipo de nutriente, a matriz na qual o nutriente está localizado e as ligações moleculares desse nutriente. No que diz respeito aos fatores extrínsecos, destacam-se: a quantidade desse nutriente na dieta e as interações que ele pode facilitar, os fatores de atenuação da biotransformação, o estado nutricional do indivíduo e os fatores genéticos associados ao indivíduo (COZZOLINO, 2005; SANDSTORM, 2001).

Por outro lado, para que os aminoácidos provenientes de fontes proteicas sejam metabolizados, é indispensável o processo de digestão química das proteínas dos ingredientes. Existem fatores que podem influenciar a biodisponibilidade dos aminoácidos dos ingredientes tais como condições de processamento, presença de anti-nutrientes, composição química e física da proteína e conteúdo de fibra do ingrediente (Sauer & Ozimek, 1986; Sibbald, 1987); isto permite que as matrizes nutricionais das rações sejam ajustadas e suplementadas com aminoácidos cristalinos com digestibilidade assumida de 99%, porém com biodisponibilidade relativa diferente entre as fontes de aminoácidos cristalinos.

Com testes de dose-resposta, é possível avaliar a biodisponibilidade relativa de precursores nutricionais e, a aplicação posterior de regressão linear ou não linear aos dados permite avaliar a biodisponibilidade das diferentes fontes do ingrediente teste, que geralmente é expressa em porcentagem. A utilização da regressão linear ou não linear dependerá da resposta e do ajuste dos dados em cada modelo, neste caso da resposta da variável dependente sob estudo à diferentes níveis de ingestão ou suplementação de um precursor de nutriente, como foi evidenciado por Vieira et al. (2004).

Devido à abordagem estatística envolvida no estudo da biodisponibilidade de precursores de nutrientes, certos requisitos estatísticos devem ser atendidos quando usa a regressão de dados. Na regressão linear (Equação 1), a resposta deve ser linear em relação à fonte de nutrientes, o intercepto deve ser o mesmo e a resposta ao nível de adição zero deve ser igual ao intercepto, mas essas condições podem não ser atendidas devido à variabilidade dos dados (LITTELL *et al.*, 1997).

$$y = a + b * (1 - e^{-c1 * (L-TRP\ 98\% + c2 * L-TRP\ 60\% + c3 * L-TRP\ 98\% - TRP)})$$

Equação 1

A análise de regressão examina a relação entre uma variável, chamada variável dependente, ou outras variáveis, chamadas variáveis independentes. Essa relação é representada por um modelo matemático que relaciona a variável dependente com as variáveis independentes. Este modelo é chamado de regressão linear simples quando uma relação linear é definida entre as variáveis independentes, e quando múltiplas variáveis independentes são incluídas, o modelo é chamado de regressão linear múltipla. A regressão linear simples e a regressão linear múltipla (Equação 2) são suficientes para determinar a bioeficiência das fontes de metionina em comparação com o padrão (LEMME, 2002).

$$y = a + b * (L - TRP\ 98\% + c1 * L - TRP\ 60\% + c2 * L - TRP\ 98\% - TRP)$$

Equação 2

2.7. Método Dose Resposta

O método dose resposta consiste na determinação das exigências nutricionais com base nas respostas de desempenho dos animais submetidos a níveis crescentes do nutriente nas dietas SAKOMURA & ROSTAGNO, (2016). Ele caracteriza as interações traçando uma curva resposta em função de um nutriente limitante na dieta em determinado período (MERCER et al., 1989).

Segundo Sakomura & Rostagno (2016), a partir desse ponto, observa-se uma fase de estabilização no crescimento e, posteriormente, dependendo do nutriente, pode ocorrer uma diminuição no peso do animal (Figura 3). O processo decorrente do aumento gradual da inclusão de um nutriente na dieta, partindo de níveis baixos até níveis elevados, pode ser dividido em quatro fases distintas:

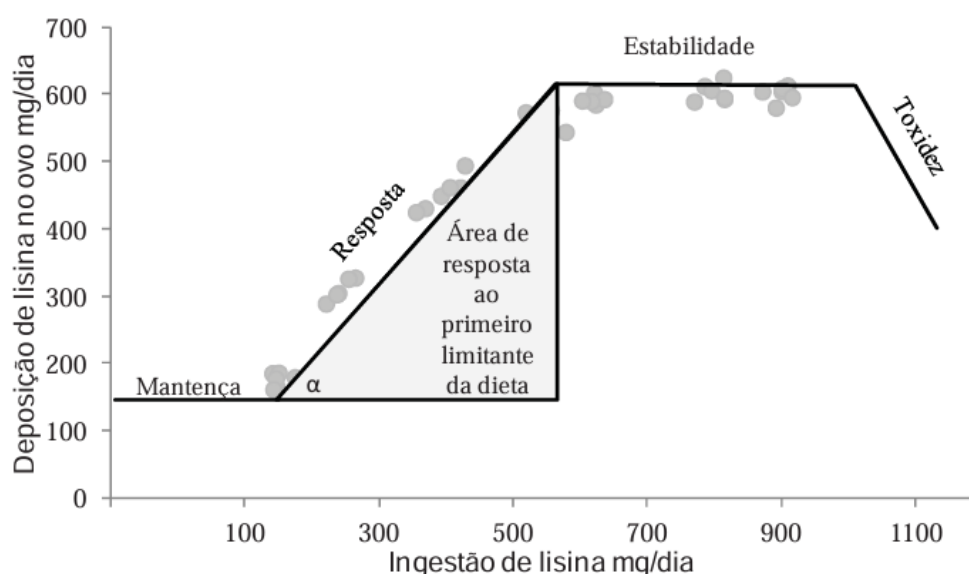


Figura 3. Curva resposta de poedeiras comerciais submetidas a dietas limitantes em lisina digestível (Sakomura & Rostagno, 2016).

a) Fase inicial: Nesta etapa, o aumento do nutriente garante apenas a sobrevivência do animal (manutença), uma vez que os níveis são insuficientes para promover o crescimento.

b) Fase de resposta: Os animais começam a apresentar crescimento e uma melhor eficiência alimentar, até alcançar um ponto onde a produção se estabiliza.

c) Fase estável: Nesta fase, os níveis do nutriente não resultam em aumento da produção nem em toxicidade devido ao excesso.

d) Fase tóxica: Um nível elevado do nutriente pode ocasionar uma redução na produção devido a efeitos como interações ou antagonismos (Figura 3).

É importante considerar que as respostas fisiológicas são resultantes do complexo multifatorial das interações entre organismo e fatores ambientais, incluindo dieta. Segundo Mercer et al. (1989; 1993), embora as vias metabólicas expliquem as respostas fisiológicas mediante mudanças na concentração do nutriente limitante na dieta, é desejável dispor de modelos matemáticos que descrevam com precisão essa relação, ou seja, o modelo deve ter significado matemático e biológico.

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CAPÍTULO 2. Bioavailability of Tryptophan Biomass for Laying Hens

Este capítulo corresponde ao artigo científico publicado na revista *Animals*, 15:866, 2025.

Bioavailability of Tryptophan Biomass for Laying Hens

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ABSTRACT

Two concurrent assays were conducted with the objective of evaluating the bioequivalence and determining the relative bioavailability value (RBV) of L-tryptophan biomass 60% for laying hens. In both assays, L-tryptophan 98% was used as the reference source. A total of 216 hens were used in the two assays. The first assay was performed using the direct comparison method to assess bioequivalence between the sources. In the second assay, the slope ratio method was used to determine the RBV. The variables analyzed were feed intake, tryptophan intake, egg production, egg weight, daily egg mass, feed conversion ratio, feed efficiency, yolk weight, shell weight, albumen weight, albumen height, eggshell thickness, Haugh unit, body protein weight, body lipid

weight, body water weight, and body ash weight. The data from both assays were submitted to analysis of variance, and we only applied linear regression to variables from the data obtained using the slope ratio method that were significant at 0.05. The results obtained via the direct comparison method showed no significant difference ($p > 0.05$) between the L-tryptophan sources when using ANOVA. In the slope ratio method, the treatments only affected the egg production and daily tryptophan intake responses of the hens and linear equations were adjusted. No differences ($p > 0.05$) were found for egg quality. When we applied the slope ratio in adjusted equations, we found that the RBV = 100%. The results showed that the utilization of L-tryptophan biomass 60% in the diet of laying hens was bioequivalent.

Keywords: bioequivalence; direct comparison method; egg production; slope ratio method

1. Introduction

Tryptophan was considered a physiologically essential amino acid by Willcock and Hopkins [1], who observed that the survival and welfare of animals (rats) improved when they received tryptophan supplementation in their diets [1]. Ten years later, tryptophan was also identified as an essential dietary component [2]. In subsequent decades, studies have been carried out on the bioavailability of synthetic compounds in different isomers (D and L) and racemic mixtures (DL) of tryptophan, as well as on the additional radicals introduced into amino or carboxyl groups, such as acetyl-, benzoyl-, methylene, and hydrochloride, among others [3–5], which are used as stabilizers of the molecule to support industrial production.

Studies on tryptophan requirements (REQ) for birds began in the 1940s with the White Leghorn [6], and only in the 1950s and the 1960s were some studies carried out with laying hens using practical diets based on corn gluten [7] and corn–soybean meal [8]. According to a review conducted on journals, there was a gap of two decades (1970–1989) in the scientific production of tryptophan for commercial laying hens, ending with the results of [9]. In the last two decades, seven studies have been published on tryptophan REQ [10–16]; 70% of these studies used white laying hens, and 30% used brown laying hens,

corresponding to a total of two publications [12,14]. In general, the reviewed studies on REQ from the past five decades used a single source of supplemental tryptophan, L-tryptophan (98%), explaining the lack of studies on the bioavailability of tryptophan sources during this period.

Recently, alternative amino acid sources have been developed for animal feed [17]. The difference between these sources is the amount of biomass contained in the product, which varies from 25% for threonine [17] and 30% for valine [17] to 40% for tryptophan [17,18]. L-tryptophan biomass is produced through fermentation and granules are formed after steam heating [17]. The biomass, which corresponds to approximately 40%, contains protein, essential amino acids, organic acids, and carbohydrates [17]. Therefore, tests must be conducted to precisely measure the relative bioequivalence of crystalline sources traditionally used in the diet. It is important to study new sources of amino acids so that nutritionists have new options to use in the formulation of poultry feed.

The bioavailability of a new source is a crucial aspect of nutrient utilization, which encompasses digestion, absorption, metabolism, and the utilization of nutrients [19–21]. This is because advances in biotechnology are constant, with the development of more efficient strains in the production of L-tryptophan, such as the use of *Corynebacterium* [17] and *Escherichia* recently developed by Hou et al. [22].

Therefore, studies evaluating the responses of birds to significant biomass contents and determining the potential of the amino acid biomass for each poultry segment are needed. In this context, recent studies on broiler chickens and pigs have evaluated their growth [17,18]; however, studies on laying hens are required.

Recent studies using only ANOVA have shown that biomass amino acid sources are equivalent to traditional crystalline amino acid sources, confirming the similarity between sources to ensure animal performance [17,18]. Our study hypothesizes that L-tryptophan biomass (60%) has a relative bioavailability (RBV) comparable to traditional crystalline L-tryptophan sources and that its inclusion in the diet of Hy-Line Brown laying hens will not negatively affect egg production, egg quality, and body composition. However, further studies are needed to determine the RBV of L-tryptophan biomass 60%. Therefore, this

study aimed to evaluate the effect of RBV on production results, the composition and quality of eggs, and the chemical composition of the bodies of commercial Hy-Line Brown laying hens.

2. Materials and Methods

2.1. Ethical and Study Approvals

Two studies were performed using laying hens brown at the facility of the São Paulo State University. All experimental procedures were approved by the Ethics Committee for the Use of Animals under protocol number 3340/22.

2.2. Assay 1: Direct Comparison Method

2.2.1. Housing, Management and Experimental Design

A total of 66 laying hens (60 weeks old) of the Hy-Line Brown variety, with an average body weight of 1763 ± 0.10 kg and egg production, were housed in experimental facilities (open shed) in a two-tier cage system. The experimental unit consisted of individual cages equipped with linear feeders and nipple drinkers. The hens had free access to water, and feed was provided twice a day, totaling 110 g per hen. The experiment lasted 119 days, with an additional 15-day adaptation period, totaling 133 days. The lighting program used during the experiment consisted of 16 h of fluorescent light at an intensity of 30 lux, measured using a lux meter. The average temperature was 23.1 ± 2.0 °C. The experimental design was completely randomized, with two treatments and 11 replicates of three hens, totaling 22 experimental units. Daily management was performed according to the brown Hy-Line guidelines.

2.2.2. Treatments

The amino acid and energy contents of corn and soybean meal were determined using near-infrared spectroscopy and adjusted in the formulations of the experimental diets. Treatments 1 and 2 were formulated to contain 0.172% digestible tryptophan and met the ideal ratio of tryptophan to lysine of 23% [23]. Treatment 1 consisted of supplementation with 1.0 g/kg of L-tryptophan biomass 60%. Treatment 2 involved supplementation with 0.612 g/kg L-tryptophan (98%). The experimental diets were formulated according to

nutritional recommendations of [23]. The chemical compositions of the diets are shown in Table S1.

2.3. Assay 2: Slope Ratio Method

2.3.1. Animals, Experimental Conditions, and Environment

At 59 weeks of age, 150 Hy-Line Brown laying hens were selected based on their body weight (1730 ± 0.09 kg) and egg production. For maximum uniformity, egg production in each experimental unit was monitored two weeks before the beginning of the study. Thereafter, the hens (61 weeks old) were housed in experimental facilities in metabolic cages (45 L cm $\times$ 50 W cm $\times$ 45 H cm), with stocking density (750 cm²/hen), in a climate- controlled chamber set at 22°C and 55% humidity. Each cage was equipped with individual feeders and nipple drinkers. The experimental design was completely randomized, with five treatments and ten replicates of three hens, totaling 50 experimental units. The lighting and daily management were the same as in Assay 1. The experiment lasted for 10 weeks.

2.3.2. Treatments and Diets

Treatments consisted of five experimental diets in mash form. The basal diet (BD; Treatment 1—T1) was formulated to contain 0.125% digestible tryptophan without crystalline L-tryptophan supplementation (Table 1). Treatments 2 and 3 (T2 and T3) were formulated to contain 0.145% and 0.165% digestible tryptophan (supplemented with L-tryptophan 98%, L-TRP 98%), respectively, whereas treatments 4 and 5 (T4 and T5) were formulated to contain 0.145% and 0.165% digestible tryptophan (supplemented with L-tryptophan biomass 60%, L-TRP 60%), respectively. The levels of tryptophan in the diet (0.00, 0.145, and 0.165%) were used to obtain a response curve to define the bioequivalence of different dosages of L-tryptophan (Table 2).

Table 1. Composition (kg/ton) of the basal diet used in the assay.

Ingredients	Basal Diet
Grain corn	699.701
Soybean meal 46 of CP% ¹	146.225
Meat and bone meal 42 of CP% ¹	49.222
Coarse limestone	15.193
NaCl	75.393
Sodium bicarbonate	3.086
L-Met 100	2.371
L-Lysine	1.753
L-Threonine	0.648
L-Valine	0.376
L-Isoleucine	0.781
Choline chloride 60% of ChoCl ²	0.750
Inert (Kaolin)	2.501
Premix ³	2.000
Total	1000

¹ Crude protein; ² choline chloride; ³ content per kg of diet: folic acid—1.232 mg; pantothenic acid—26.40 mg; biotin—0.0352 mg; niacin—61.60 mg; vitamin A—4.224 mg; vitamin B1—3.872 mg; vitamin B12—0.02112 mg; vitamin B2—10.032 mg; vitamin B6—5.632 mg; vitamin D3—0.1232 mg; vitamin E—23.584 mg; vitamin K3—3.52 mg; copper—16.00 mg; iron—100.00 mg; iodine—2.40 mg; manganese—140.00 mg; selenium—0.60 mg; zinc—140.00 mg.

Table 2. Treatment's description used in the assay.

Items	T1	T2	T3	T4	T5
	B	L-	L-	L-	L-
	D ¹	TRP 98% ²	TRP 98% ²	TRP 60% ³	TRP 60% ³
Basal	997.499	997.499	997.499	997.499	997.499
Inert (Kaolin)	2.501	2.297	2.093	2.193	1.886
L-Tryptophan 98%	-	0.204	0.408	-	-
L-Tryptophan biomass 60%	-	-	-	0.308	0.615
Total	1000.000	1000.000	1000.000	1000.000	1000.000

¹ Basal diet; ² L-Tryptophan 98%; ³ L-Tryptophan biomass 60%.

The ingredients, except for inert and L-tryptophan, were premixed for 5 min to prepare the BD and BD + L-tryptophan treatments. L-tryptophan and inert were added in appropriate proportions (Table 2) to the premixed solution to form the treatments and remixed for 5 min in a horizontal mixer. Only inert was added to treatment 1 (BD).

The BD was made prior to the ones containing L-tryptophan to avoid cross- contamination. Diets did not contain any enzymes, antibiotics, or growth promoters. The nutritional level (Table 3) of experimental diets was formulated according to [23].

Table 3. Calculated and analyzed compositions of the experimental diets.

Items	T1	T2	T3	T4	T5
	BD ¹	L- TRP 98% 2	L- TRP 98% 2	L- TRP 60% 3	L- TRP 60 ³
Calculated					
crude protein, %	15.2	15.2	15.2	15.2	15.2
Metabolizable energy, kcal/kg	2800	2801	2803	2801	2803
Lysine, %	0.730	0.730	0.730	0.730	0.730
Methionine + cysteine, %	0.660	0.660	0.660	0.660	0.660
Threonine, %	0.510	0.510	0.510	0.510	0.510
Tryptophan, %	0.125	0.145	0.165	0.145	0.165
Arginine, %	0.805	0.805	0.805	0.806	0.806
Valine, %	0.640	0.640	0.640	0.640	0.640
Isoleucine, %	0.580	0.580	0.580	0.580	0.580
Potassium, %	0.519	0.519	0.519	0.519	0.519
Sodium, %	0.160	0.160	0.160	0.160	0.160
Calcium, %	4.180	4.180	4.180	4.180	4.180
Available phosphorus, %	0.360	0.360	0.360	0.360	0.360
Total choline, mg/kg	1260	1260	1260	1260	1260
Linoleic acid, %	1463	1464	1464	1464	1464
Analyzed					
crude protein, %	15.88	15.94	15.95	16.06	16.07
Tryptophan ⁴ , %	0.126	0.146	0.166	0.146	0.166

¹ Basal diet; ² L-Tryptophan 98%; ³ L-Tryptophan biomass 60%; ⁴ total tryptophan × digestibility coefficient from Rostagno et al. [23].

2.4. Performance

The same procedures—direct comparison method and slope ratio method—were used in both assays to collect data. The hens were fed 110 g of feed daily at the same time each morning. At the end of each week, the leftovers were weighed to quantify weekly feed intake. Body weights were measured at the beginning and end of the assay. Egg production and mortality were recorded daily, and egg weight was measured weekly for three consecutive days. Egg mass was calculated as the average egg weight multiplied by egg production for each treatment in each period, divided by 100. The weekly leftovers per replicate were weighed to calculate feed intake and feed conversion ratio per egg mass. We corrected the feed conversion based on mortality. The variables analyzed were feed intake (FI, g/day), body weight

(BW, g), egg production (EP, %), egg weight (EW, g), egg mass (EM, g/hen/day), and feed conversion ratio (FCR, g/g) by egg mass, corrected for mortality.

2.5. Egg Assessment

The same procedures—direct comparison method and slope ratio method—were used in both assays to collect data. Shell-less eggs, those with soft shells, and dirty eggs were recorded daily. At the end of the assay, 2 eggs per experimental unit, totaling 22 eggs per treatment, were randomly selected for egg quality analysis. The eggs were evaluated for weight, albumen height, Haugh unit, yolk weight, and eggshell thickness. The eggs were weighed and broken, and the yolk was separated from the albumen and shell. The shells were washed and air-dried for 48 h, and after weighing them, their eggshells' thickness was measured. Albumen weight was defined as the difference between the egg's weight and the sum of the yolk and shell weights. Egg, yolk, and eggshell weights were determined using an analytical balance. The eggshell thickness and albumen height were measured using Vernier calipers. The Haugh unit was calculated as previously described [24].

2.6. Body Composition

Using only the direct comparison method, a hen with a body weight matching the average of each treatment replicate was selected for body composition analysis using dual-energy X-ray absorptiometry (DEXA, Hologic-QDR® model 13.4.2., Bedford, MA, USA), totaling 50 animals. At the end of the assay, the hens were subjected to an 8 h solid fast to empty the digestive tract, and then they were weighed and sacrificed by cervical dislocation for the analysis of body composition. The total mass, bone mineral content, bone mineral density, fat mass, and lean mass were determined using DEXA; then, the protein, lipid, water, and ash weights were obtained based on the equations of Alves et al. [25]. The calculations were realized as follows: deposition = body weight × protein or lipid or water or ash content.

2.7. Chemical Analysis

The amino acid contents of corn and soybean meal were determined using near-infrared spectroscopy and adjusted in the formulations of the

experimental diets. The total nitrogen in the diets was determined using the Kjeldahl method, with a factor of 6.25 used for conversion into protein.

2.8. Statistical Analysis

In both assays, the data were tabulated and analyzed for assumptions of the homoscedasticity of variance (Brown–Forsythe test) and normality of errors (Cramér–von Mises test) using a mixed model. The experimental unit was considered a random effect, and the treatments were considered a fixed effect. The F-test was used for ANOVA, and regression analysis was conducted for the slope ratio method assay when the null hypothesis was rejected ($p \leq 0.05$ level of significance).

The RBV of L-tryptophan 60% was estimated using slope ratio analysis, as described by [21], according to the linear model $\xi = \alpha + \beta_s X_s + \beta_t X_t \pm e$, where ξ is the value observed; α is the intercept; e is random error; β_s and β_t are the slopes for L-tryptophan 98% and L-tryptophan biomass 60%, respectively; and X_s and X_t are the concentrations of L-tryptophan 98% and L-tryptophan biomass 60% in the diet, respectively. For the nonlinear model, $\xi = \alpha + \delta \times (1 - e^{-(\beta_s X_s + \beta_t X_t)}) \pm e$, where ξ , α , e , β_s , β_t , X_s and X_t are defined.

The NLMixed model procedure was used for random-effect linear and nonlinear regression analyses. The α was randomized in both models. The r^2 value was obtained from the ratio between the sq model and sq treatment. The asymptote value is $\alpha + \delta$. Statistical analyses were performed using SAS 9.4 (Statistical Analysis for Windows; SAS Institute Inc., Cary, NC, USA).

3. Results

Assays were performed, and the results obtained using the applied methods, direct comparison, and the slope ratio are presented below.

3.1. Assay 1: Direct Comparison Between L-Tryptophan Biomass 60% and L-Tryptophan 98%

An assessment of 60% L-tryptophan biomass was performed, focusing on the productive performance responses of brown laying hens. These

variables were used to evaluate the bioequivalence of L-tryptophan sources over 17 weeks in hens fed a non-limiting diet. Direct comparison was performed between the two sources of L-tryptophan (60% L-tryptophan biomass and 98% L-tryptophan) using a control diet containing 0.172% tryptophan (Table 4). This comparison revealed a numerical superiority of 2.2% in egg production (EP) and 3.7% in egg mass for the diet supplemented with L-tryptophan biomass 60%, although these differences were not statistically significant ($p > 0.05$).

Table 4. Average responses to performance, body composition, and egg quality from brown laying hens fed with two non-limiting diets formulated with two sources of L-tryptophan.

Variables	TRP 60% 1	TRP 98% 2	Average	Stderr ³	p-Value	CV, % ⁴
Feed intake, g/day hen	108.7	109.2	109.0	0.2	0.125	0.7
Tryptophan intake mg/day hen	186.9	187.9	187.4	0.3	0.125	0.7
Egg production, %	94.1	92.5	93.3	0.5	0.128	2.7
Egg weight, g	59.8	59.4	59.6	0.3	0.447	2.5
Egg mass, g/day hen	56.3	54.9	55.6	0.5	0.115	3.8
Feed conversion ratio g/g	1.932	1.990	1.961	0.017	0.085	4.1
Feed efficiency, g/kg	518.5	502.9	510.7	4.5	0.083	4.1
Change body weight, g/day hen	1.3	1.0	1.2	0.1	0.181	55.8
Body weight, kg	1.9	1.9	1.9	0.0	0.531	4.8
Body water, g	917.3	938.6	928.0	6.6	0.105	3.2
Body protein, g	272.5	278.8	275.6	1.9	0.106	3.2
Body lipid, g	186.7	171.9	179.3	2.1	<0.0001	5.2
Body ash, g	56.7	58.1	57.4	0.4	0.098	3.2
Yolk weight, g	15.5	15.1	15.3	0.1	0.198	3.7
Eggshell weigh, g	5.9	6.0	6.0	0.1	0.650	4.6
Albumen weight, g	38.4	38.2	38.3	0.3	0.767	3.3
Eggshell thickness, μ m	398.9	405.3	402.1	3.0	0.290	3.4
Haugh unit, %	95.2	95.8	95.5	0.7	0.677	3.6
Yolk percentage, %	25.9	25.5	25.7	0.2	0.300	3.2
Shell percentage, %	9.9	10.1	10.0	0.1	0.420	4.4
Albumen percentage, %	64.2	64.4	64.3	0.2	0.706	1.6

¹ L-Tryptophan biomass 60%; ² L-Tryptophan 98%; ³ Stderr: standard error of mean; ⁴ coefficient of variation.

Additional analyses revealed no differences ($p > 0.05$) in daily feed intake, tryptophan intake, egg weight, feed conversion ratio, feed efficiency, change body weight, body weight, body water, body protein, body ash, yolk weight, eggshell weigh, albumen weight, eggshell thickness, Haugh unit and yolk, eggshell, and albumen percentage variables among laying hens fed the same nutritional levels, L-tryptophan biomass 60%, and L-tryptophan 98%

(Table 4). Among the body composition variables (Table 4), only body lipids showed a significant effect ($p < 0.05$); hens fed L-tryptophan 60% had 14.8 g more body fat. This corresponded to an increase of 8.6% compared to the level in hens fed L-tryptophan 98%.

3.2. Assay 2: Slope Ratio Method to Determine the RBV of L-Tryptophan Biomass 60%

Initially, the data were analyzed using the F-test, and the results showed no significant differences ($p > 0.05$) between the treatments for all of the variables analyzed, as shown in Tables 5 and 6.

Table 5. Average responses to treatments for daily feed intake (FI, g/hen), egg weight (EW, g), daily egg mass (EM, g/day), feed conversion ratio (FCR, g/g), and feed efficiency (FE, g/kg) of brown laying hens from 61 to 70 weeks of age.

Treatments	L-Trp ⁴	FI	EW	EM	FCR	FE
T1—BD ¹	0.125	107.2	60.0	53.0	2.04	493.0
T2—TRP 98% ²	0.145	107.7	60.7	55.0	1.96	511.0
T3—TRP 98% ²	0.165	109.2	60.0	55.5	1.97	509.0
T4—TRP 60% ³	0.145	108.9	59.4	54.6	1.99	503.0
T5—TRP 60% ³	0.165	108.7	61.5	56.8	1.93	520.0
Average		108.3	60.3	55.0	1.98	0.507
Stderr ⁵		0.3	0.3	0.5	0.02	0.004
p-value		0.182	0.365	0.160	0.390	0.339
CV, % ⁶		0.193	3.68	5.81	5.57	5.53

¹ Basal diet; ² L-Tryptophan 98%; ³ L-Tryptophan biomass 60%; ⁴ level of L-tryptophan; ⁵ standard error of mean;

⁶ coefficient of variation.

Table 6. Average responses to treatments in terms of yolk weight (YW, g), shell weight (SW, g), albumen weight (AW, g), albumen height (AH, mm), eggshell thickness (EST, μ m), and Haugh unit (HU, %) of brown laying hens from 61 to 70 weeks of age.

Treatments	L-Trp ⁴	YW	SW	AW	AH	EST	HU
T1—BD ¹	0.125	15.1	5.9	38.0	9.7	406.8	98.1
T2—TRP 98% ²	0.145	15.1	6.2	38.3	9.9	403.6	99.3
T3—TRP 98% ²	0.165	14.9	6.0	38.7	9.7	398.0	97.7
T4—TRP 60% ³	0.145	14.8	6.2	39.0	9.9	411.8	97.5
T5—TRP 60% ³	0.165	15.0	6.1	39.2	10.1	408.7	99.4
Average		15.0	6.1	38.6	9.9	405.8	98.4
Stderr ⁵		0.1	0.04	0.3	0.1	2.2	0.4
p-value		0.765	0.129	0.599	0.607	0.332	0.491
CV, % ⁶		3.27	3.95	4.37	6.05	3.75	3.05

¹ Basal diet; ² L-Tryptophan 98%; ³ L-Tryptophan biomass 60%; ⁴ level of L-tryptophan; ⁵ standard error of mean; ⁶ coefficient of variation.

For egg quality (Table 6), the eggshell weight was 0.23 g higher than that found for the BD treatment, corresponding to 3.8% improvement. However, it was not significant ($p = 0.1256$) based on the BD (T1—0.125% L-Trp) compared to that in the other treatments.

The treatments only affected the daily EP and daily tryptophan intake responses of the hens and allowed the fitting of the linear equations (Table 7). In the treatment without the supplementation of L-tryptophan sources (level at 0.125%—L-tryptophan), the daily EP reached was 86.6% with a daily tryptophan intake of 134 mg/hen.

Table 7. Average responses of daily tryptophan supplementary intake (Trp Intake, mg/hen), daily intake of tryptophan supplemental corrected for source concentration (Trp Intake Corrected, mg/hen) and daily egg production (EP, %) of brown laying hens from 61 to 70 weeks of age.

Treatments	L-Trp ⁴	Trp Sup ⁵	Trp Intake	Trp Intake Corrected	EP
T1—BD ¹	0.125	0	134.0	0	84.8
T2—TRP 98% ²	0.145	0.204	156.2	21.5	90.7
T3—TRP 98% ²	0.165	0.408	180.2	43.7	92.3
T4—TRP 60% ³	0.145	0.333	157.7	21.7	91.0
T5—TRP 60% ³	0.165	0.667	179.4	43.5	92.1
Average			161.5	26.1	90.7
Stderr ⁶			2.5	0.5	0.7
p -value			<0.0001	<0.0001	0.041
CV, % ⁷			1.85	2.05	4.97

¹ Basal diet; ² L-Tryptophan 98%; ³ L-Tryptophan biomass 60%; ⁴ level of L-tryptophan (%); ⁵ L-tryptophan supplementary in the diet (g/kg); ⁶ standard error of mean; ⁷ coefficient of variation.

At a level of 0.145%, laying hens ingested 21.5 mg/hen of daily intake of supplemental tryptophan, corrected by the source, with L-tryptophan 98%, and 21.7 mg/hen of daily intake of supplemental tryptophan, with L-tryptophan biomass 60%. EP reached 90.7% with L-tryptophan biomass 98% and 92.0% using L-tryptophan biomass 60%. Similarly, at a nutritional level of 0.165%, the daily intake of supplemental tryptophan, corrected by the source, was 43.7 mg/hen with L-tryptophan 98% and 43.5 mg/hen with L-tryptophan biomass 60%, while EP achieved 92.3% and 92.01%, respectively. It is noteworthy that the daily intake of tryptophan increased linearly with an increase in the nutritional supplement levels of both sources of L-tryptophan.

A simple two-group model was used to test regression curves. The regression of the EP variable on TrpSup was assumed (Table 7). The variables

were measured in hens, grouped according to the L-tryptophan source. We sought to test two hypotheses to answer this question:

- (a) Whether sources of L-tryptophan have separate regression curves;
- (b) Whether there is a difference in regression slopes for L-tryptophan sources.

The results of the analyses of the intercept and slope hypotheses provide insights into the relationships between these variables. The intercept (90.2°) represents the point where the equation line crosses the vertical axis (EP) when the supplemental tryptophan (TrpSup, horizontal axis) is zero. However, an estimated difference of -5.44 for the intercept between the BD (84.8) and L-tryptophan sources was observed. This difference was significant ($p = 0.025$), suggesting differences in EP in the absence of tryptophan supplementation. The interaction between the slope (3.18) of the equation and the source of L-tryptophan indicates whether there are variations in the responses to EP according to the different sources of L-tryptophan in the diet. This study showed no significant interaction ($p = 0.614$), indicating that the relationship between tryptophan intake and EP did not differ significantly between L-tryptophan sources. The hypothesis that the dependent variable, EP, is explained by a single model when TrpSup changes was accepted.

This assay was performed to determine the RBV using the slope ratio method with L-tryptophan biomass 60% relative to the source L-tryptophan 98% for brown laying hens. The relationship between the daily intake of supplemental tryptophan corrected for source concentration and the EP for each tryptophan source resulted in the equations shown in Table 8.

Table 8. Averages of the estimated parameters, standard errors (SEs), and fit statistics of the models, which were fitted to describe the relationship between daily egg production (%) at different daily levels of tryptophan intake supplementary (mg/hen) and the relative bioavailability value (RBV) of brown laying hens from 61 to 70 weeks of age.

Models	Parameter Estimates				RBV		
	Estimate	SE	Lower	Upper	Lower	Average	Upper
Linear	α	86.3	1.3	83.4	89.1		
AIC = 274.5	β_s	0.153	0.045	0.050	0.256	98.0	100.4
$R^2 = 91.0\%$	β_t	0.151	0.044	0.050	0.251		
Nonlinear	α	84.8	1.5	81.5	88.2		
AIC = 273.4	δ	8.3	2.3	3.0	13.5	101.0	107.0
$R^2 = 91.0\%$	β_s	-0.055	0.039	-0.144	0.033		
	β_t	-0.055	0.040	-0.146	0.035		

α is the intercept. The asymptote value is $\alpha + \delta$. β_s is the slope for L-tryptophan 98%; β_t is the slope for L-tryptophan biomass 60%.

Two models were fitted to interpret the relationship between supplemental tryptophan intake and daily EP. The estimated parameters are presented in Table 8. For the linear model ($EP = 86.3 + 0.153X_s + 0.151X_t$, AIC = 247.5), the RBV was calculated as the relationship between the slopes $0.151 \div 0.153 = 98.5\%$. Considering a 95% confidence interval, the lower and upper limits were calculated at 98% and 100%, respectively, resulting in an overall mean of 99%. For the nonlinear model ($EP = 84.8 + 8.3(1 - e((-0.055X_s - 0.055X_t)))$, AIC = 273.4), the RBV was calculated as the relationship between the slopes $0.0553 \div 0.0553 = 100\%$. Considering a 95% confidence interval, the lower and upper limits were calculated at 101% and 107%, respectively, resulting in an overall mean of 103%.

Figure 1 shows the lines predicted by each model and the observed treatment means. Differences were observed between the models in relation to the origin of the predicted curve (Figure 1). The nonlinear model coincided with the average response to the BD (84.8%).

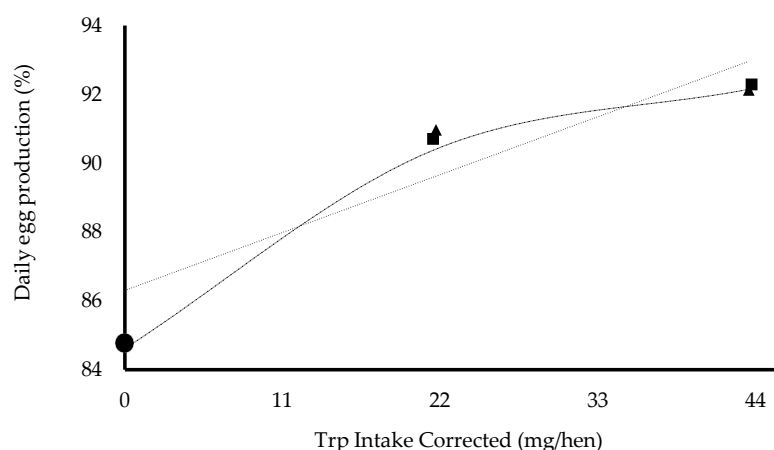


Figure 1. The relationship between daily tryptophan intake supplementation, corrected for source concentration (Trp Intake Corrected) and daily egg production. The bioavailability of L-tryptophan biomass 60% compared to L-tryptophan 98% based on egg production, presented using slope ratio linear () and nonlinear models (). , basal diet; , L-tryptophan 98%; , L-tryptophan biomass 60%.

4. Discussion

This study was conducted to evaluate whether the L-tryptophan biomass 60% source is bioequivalent to L-tryptophan 98% and to determine the bioavailability of tryptophan in the L-tryptophan biomass 60% source. Two assays were conducted, and the data obtained allowed for statistical analysis and the extraction of the results necessary to answer questions about the bioequivalence and bioavailability of L-tryptophan biomass 60%.

The results obtained using the direct comparison method (Table 4) on tryptophan sources support the hypothesis that the sources are bioequivalent because of the similarities in the responses of laying hens. A direct comparison between tryptophan sources, using a control diet with 0.172% tryptophan (Table 4), revealed a slight numerical superiority of 2.2% in EP and 3.7% in egg mass for the diet supplemented with granular L-tryptophan, although this difference was not statistically significant ($p > 0.05$). A detailed analysis of the responses of laying hens rules out the hypothesis of the mobilization of body reserves to sustain EP, given the improvement observed in production.

A previous study conducted using this method showed that 60 sources of L-tryptophan biomass and L-tryptophan 98% were bioequivalent in terms of ensuring the zootechnical indices of broilers [18]. Also, in research by Lee et al. [18], although not significant at the limit of the margin of error, there is an

indication of the better utilization of dietary nitrogen with the supplementation of L-tryptophan biomass 60%. The results obtained in the present study for protein deposition in the bodies of laying hens did not differ between tryptophan sources, which was similar to the findings of Lee et al. [18]. However, nitrogen use in birds directly interferes with lipid metabolism [26] and its utilization rate [27]. In the direct comparison test, the only significant difference ($p < 0.0001$) found between tryptophan sources was an increase in lipid deposition in the bodies of laying hens.

Greater lipid deposition in the body provides energy storage and depends directly on the energy expenditure of the cells demanded by protein metabolism, especially when there is excess nitrogen in the diet [22]. Thus, the hypothesis of excess nitrogen originating from biomass was not supported by the results (Table 4). If this were the case, the cell would demand a greater energy supply to excrete excess nitrogen from the body, and consequently, less energy would be available for lipid synthesis [28,29]. Therefore, there was an energy- saving effect with the use of the source L-tryptophan biomass 60%, but it was not enough to affect feed efficiency owing to its limited inclusion in the diet. The cumulative effect of daily intake of additional calories may have contributed to increased lipid deposition in the bodies of laying hens. In this sense, it is not possible to rule out the hypothesis that the energy value of the L-tryptophan biomass 60% source was underestimated. The metabolizable energy value of 4.288 kcal/kg of the L-tryptophan biomass 60% source was obtained using equation [23]. Therefore, additional studies are needed to confirm the findings of this research and to measure the real supporting contribution of biomass to the animal response.

A combination of methods allows for a more comprehensive and reliable assessment of nutrient availability and provides a solid basis for developing diets that optimize bird performance and health. In the second assay, the RBV was determined using the slope ratio method, and the condition of this method was the relationship between the dose and the hen's response to ensure the accuracy and reliability of the RBV [21]. Within the sample space defined in this study (Tables 5 and 6), only EP and tryptophan intake were significant, which allowed the application of the procedure to determine the RBV of tryptophan.

The other variables were not affected by the treatment. Therefore, 0.125% tryptophan in the diet or 134 mg/hen day (Table 7), which corresponds to a reduction of approximately 18% in relation to the recommendation (163 mg/day) of the guidelines, was not sufficient to trigger signs of deficiency in the other variables for an experimental period of 10 weeks, making it impossible to use these variables to determine the RBV.

Egg production was the variable most sensitive to tryptophan levels, as the dose of 0.125% tryptophan in the diet reduced its value after 10 weeks. This result supports the finding of [30], who described how the main response is severely affected by the restriction of amino acid intake, while egg weight rarely decreases. Its greatest reduction has been close to 10%, regardless of how inadequate the amino acid intake is [30–32]. Therefore, future studies should use a greater degree of deficiency over a longer period to observe the effects of tryptophan deficiency on other variables, especially those linked to egg quality.

The adaptation period is a problem in research with hens. When the degree of deficiency is small, these hens mobilize body reserves to maintain reproduction, according to previous studies [31,33]. In this research, the 10-week period is traditionally used [31–35]. Among the available information, the tryptophan recommendations ranged from 186 mg/day [23] to 249 mg/day [14], generating a degree of deficiency between 28% and 46%, respectively. However, the results obtained suggest that the requirement for tryptophan is less than 186 mg/day; consequently, the degree of deficiency was less than 28%. This justifies the reduction of only 6 percent units in egg production, which was the result obtained for the basal diet in the evaluated period; therefore, future studies should consider a period longer than 10 weeks to observe effects on the other variables, especially egg quality.

The relationship between the daily intake of supplemental tryptophan, corrected for source concentration, and the EP for each tryptophan source resulted in the equations shown in Table 8. The RBV was determined using linear and nonlinear models, and the results obtained were 99% and 103% of RBV, respectively, for L-tryptophan biomass 60%, relative to L-tryptophan 98%, resulting in an overall mean of 101%. However, the selection of the model to conclude the recommendation was based on the lowest AIC value; therefore, following this criterion, the RBV was equal to 100% for L-tryptophan biomass

60%. This value is the result of the similarity found in the adjusted values for the slope (β_t and β_s) of the tryptophan sources ($0.055 \div 0.055 = 1$).

Analyzing the quality of the adjustment following the recommendation of Silva et al. [36], the errors associated with the mean value of β_t and β_s were more significant than those found for the linear adjustment approximately by 2.4 times, and this may have affected the estimation of the confidence interval, which recommends a lower limit very close to the mean value found. Other studies on bioavailability in laying hens have also emphasized the importance of errors in estimating confidence intervals [37,38].

The importance of errors in estimating the confidence interval was corroborated by the values obtained using the linear model. In this model, the errors were smaller, and the confidence interval was symmetrical to the determined mean value of the RBV. A linear model was not selected due to its adjustment to the hen's responses. From Figure 1, it can be verified that the adjustment of the exponential curve to the observed means is better, regardless of the adjustment and selection statistics. The greater number of parameters in the exponential model allowed for improved interpretation of the hens' responses, accurately estimating the response to the BD and the maximum response of the hens. These qualities were established by Littell et al. [21] as the criteria that must be met in the bioavailability assay.

According to [21], three assumptions must be made to ensure the validity of the slope ratio assay when using a linear model. The first is to assume linearity in the dose-response relationship, the second is to assume the same intercept between the fitted lines, and the third is to assume that the intercept of the line coincides with the response of the BD. Only one assumption was met by the linear model.

Considering the instantaneous derivative ($\xi_n - \xi_{n-1} / X_n - X_{n-1}$), the values between the treatments at 0.145% and 0.125% and at 0.165 and 0.145% were 0.280 and 0.068 production unit per unit of supplemental tryptophan intake (Figure 1), respectively. Therefore, the unitary response rate decreases abruptly by approximately four times between the levels of 0.165% and 0.145%, and the derivative should be approximately constant to be linear. The intercept of the supplement levels is the same between sources, according to Eq.: $EP = 90.2 + 3.18\text{TripSup}$, but it does not coincide with the BD. For this reason, the expo-

nential model ($EP = 84.8 + 8.3(1 - e((-0.055X_s - 0.055X_t)))$, $AIC = 273.4$) was preferred to assess whether the RBV of the L-tryptophan biomass was 60%. This research confirms the position of previous studies that recommended the careful evaluation of the mathematical model used to establish the RBV [37,38].

It is important to consider the physical form of supplemental amino acids and the digestion and absorption processes of their units. The powdered physical form is most commonly used in animal feed. This form is absorbed more quickly, and for situations that require immediate availability, its use is advantageous to some extent. The physical form of tryptophan in the L-tryptophan biomass source 60% was granules and it has the characteristic of slower release, causing its release to be prolonged.

For situations that require slower release, it is, to some extent, advantageous. This is valid for hens that are normally raised with feeding controls [34]. The results obtained by [35] clearly demonstrated that the higher release speed of synthetic amino acids, and consequently, rapid absorption, can affect the egg mass production response because of the higher concentration of synthetic amino acids in the flow between the intestine, blood, and tissues available for protein synthesis. When [35] increased the number of meals, there was an improvement in egg mass production and, consequently, in the efficiency of the use of supplementary amino acids. Therefore, the physical form of tryptophan granules present in the 60% L-tryptophan biomass source may be an interesting characteristic of these hens. However, at this time, we only had one hypothesis since the speed of tryptophan passage from different sources in the gastrointestinal tracts of birds was not measured. Thus, the slower release of tryptophan in the 60% L-tryptophan biomass source was limited, supporting an explanation for the upper limit of the confidence interval, which was 107% in relation to the 98% L-tryptophan source. In this study, 100% of the 60% L- tryptophan biomass was considered digestible; therefore, the nutritional matrix assumed a concentration of 60% L-tryptophan to be fully digestible. Although L-tryptophan excretion was not measured, its bioavailability was close to 100%; therefore, excretion was not expected to be affected. This hypothesis was supported by the responses of the birds, considering that there was no reduction in the responses of laying hens to treatments with L-tryptophan biomass 60%.

5. Conclusions

No differences were found for egg quality. For body composition, only body lipids showed a significant effect. The bioavailability of L-tryptophan biomass 60% was determined at 100% and it presented the same bioequivalence as the crystalline source of L-tryptophan 98%.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ani15060866/s1>.

Table S1: Feed composition of the experimental diets (kg/ton). Table S2: Composition (kg/ton) of the basal diet used in the assay.

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Institutional Review Board Statement: This study was conducted using brown laying hens at the experimental facility of São Paulo State University (UNESP, Jaboticabal City—State of São Paulo). All procedures were approved by the Ethics Committee for the Use of Animals (CEUA) under Protocol number 3340/22.

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Conflicts of Interest: Author Bernardo Rocha F. Nogueira and Eliane Aparecida da Silva were employed by the company CJ Bio Brazil. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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CAPÍTULO 3. A REASSESSMENT OF TRYPTOPHAN REQUIREMENTS FOR AGED BROWN LAYING HENS USING AMINO ACID BIOMASS

Este capítulo corresponde ao artigo científico submetido à revista *Animals* e encontra-se em avaliação para publicação.

A Reassessment of Tryptophan Requirements for Aged Brown Laying Hens Using Amino Acid Biomass

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Simple Summary

Animal cells cannot synthesize tryptophan (Trp), and metabolic intermediates such as indole-3-pyruvic acid provide only minimal and metabolically insufficient conversion to Trp, reinforcing its classification as an essential amino acid in vertebrates. This study assessed the tryptophan (Trp) requirements of aged layers using a L-tryptophan biomass 60%. A total of 264 hens received eight Trp levels, and performance and egg quality were measured. When significant effects were found, linear-plateau and quadratic-plateau models estimated optimal intake. Trp influenced only performance variables, with ideal intake values ranging from 174.7 to 187 mg/hen per day. Based on Trp intake and its deposition in eggs, the optimal requirement was determined to be 187 mg/hen per day for maximum efficiency.

Abstract

A reassessment of the Trp requirements for brown laying hens using the Trp biomass source allows an update of the nutritional recommendation, in addition to assessing whether biomass has an adjuvant effect in the diet. This research was carried out with the aim of evaluating the efficiency utilization of Trp and determining the ideal Trp intake for aged brown laying hens using the L-tryptophan biomass 60%. A completely random design was used, with eight treatments and eleven replicates, totaling 264 hens. The variables analyzed were performance and egg quality. When the effect of Trp levels ($P \leq 0.05$) was detected, the model's linear-plateau (LP), quadratic-plateau (QP), and the first intercept of the QP in the plateau of LP were adjusted to determine the ideal Trp intake. The results obtained showed an effect of the treatments ($P < 0.05$), only on the performance variables. The first intercept obtained was 186.2, 174.7, 182.7, and 182.5 mg/hen per day for egg production, egg mass, feed conversion ratio, and feed efficiency, respectively. Based on the relationship between Trp intake and Trp deposition in the egg, the optimum Trp intake was estimated to be 187 mg/hen per day for maximum efficiency utilization of Trp by hens.

Keywords: efficiency utilization; egg production; supplementation technique

1. Introduction

The molecular structure of tryptophan (Trp) was further elucidated [1], who de-scribed its synthesis from indole-3-aldehyde. In microorganisms and plants, Trp is syn-thesized by the shikimate pathway, which is common to the biosynthesis of aromatic amino acids and uses chorismate as the final common precursor of all aromatic amino acids. Vertebrates cannot synthesize aromatic amino acids because they lack the shiki-mate pathway. That pathway uses phosphoenol-pyruvate (PEP) and erythrose-4-phosphate (E4P) as precursors to produce intermediates such as chorismate [2]. In vertebrates, although PEP can be synthesized via gluconeogenesis [3] and E4P via the non-oxidative reactions of the pentose phosphate pathway [4], there are no genes encoding 3-deoxy-D-arabino-heptulosonate-7-phosphate synthase (DAHP synthase) or chorismate

synthase. These enzymes are required to catalyze the sequential reactions that convert these substrates into chorismate [5].

The animal tissue cells do not synthesize Trp even when diets are supplemented with intermediates such as 3-indole aldehyde, L- β -3-indolelactic acid, or kynurenine [6,7]. Only supplementation with indole-3-pyruvic acid (IPA) suggesting that IPA can be metabolically converted to Trp transamination pathway [8,9]. However, subsequent studies [10] demonstrated that the transamination of IPA into Trp is limited. In rats fed Trp-free diets, IPA failed to restore serotonin (5-HT) levels or its metabolite in the cortex, and it did not prevent weight loss caused by nutritional deficiency. Thus, conversion of IPA to Trp is quantitatively insufficient to sustain serotonergic metabolism and Trp dependent functions. Reinforcing and proving the essentiality of tryptophan in vertebrates.

Although biochemical evidence of Trp essentiality [7-9], dietary supplementation of this amino acid in poultry was not established until the 1950s [11]. Since then, biotechnological processes have been developed and refined to enable the large scale production of L-tryptophan for use in animal feed, using strains of *Escherichia* or *Corynebacterium* as production organisms[12-14].

Evaluating a new source of L-tryptophan is necessary with respect to digestibility [15], bioavailability [16], and nutrient requirements, particularly for amino acids derived from biomass, given the presence of biomass in the ingredient. Nutritional requirement studies are needed because the applied methods differ from those used in earlier research in terms of dietary inclusion, experimental duration, and response interpretation. In this context, a reassessment of Trp requirements for brown laying hens using the biomass Trp source could update nutritional recommendations and evaluate whether the biomass exerts an adjuvant effect in conjunction with supplemental L-tryptophan in the diet.

Three studies with brown laying hens [17],[18],[19], recommended Trp intakes of 171, 235, and 217 mg/d, respectively. These three recommended intakes cover a 64 mg/day range, corresponding to 37 % variation, and only [17] clearly described the effect of dietary Trp limitation on egg mass response. From the efficiency of utilization data reported in these studies, a negative linear

relationship was observed ($k = 104 - 0.246 \times I$, $R^2 = 0.899$) between Trp utilization efficiency (k) and Trp intake (I). In other words, as Trp intake increased, the amount of Trp deposited did not increase. Specifically, the efficiency of Trp utilization reported by [19] was about 23% ($k = 93.6 - 0.2316 \times I$, $R^2 = 0.869$), indicating that roughly 75% of ingested Trp was not recovered as deposited egg protein and was thus allocated to other metabolic fates. In this context, researchers [18,19] have used other measures to understand hens responses to Trp intake, such as feather condition scores and stress indicators. However, these measures relate to maintenance functions, and it was not possible to assess Trp allocation to maintenance because the body weights were not reported in those studies [17-19].

Despite the importance of Trp in basal metabolism,[20] estimated its utilization efficiency for egg production at 55.4% in heavy breeder hens, a value higher than that observed in commercial brown layers. Therefore, the hypothesis that a higher Trp utilization efficiency for egg-protein deposition occurs in layers is supported by laying sequence length: breeders have shorter laying sequences and longer pauses between sequences compared to commercial layers [21]. Considering the above, this study was undertaken to evaluate Trp utilization efficiency and to determine the ideal Trp intake for aged brown laying hens using a 60% biomass L-tryptophan source.

2. Materials and Methods

2.1. Ethical and Study Approvals

A study was conducted using brown laying hens at the experimental facility of São Paulo State University (UNESP, Jaboticabal, São Paulo, Brazil). All procedures were approved by the Ethics Committee for the Use of Animals under Protocol number 3340/22.

2.2. Housing, Experimental Design and Management

A total of 264 Brown Hy-Line hens were housed in the experimental facilities, open shed in a two-tier cage system, equipped with linear feeders, nipple drinkers, and six fans. At 68 weeks of age, hens were selected based on body weight (1.760 ± 0.09 kg) and egg production ($91 \pm 2\%$) and were

distributed into 88 experimental units. The egg production from each experimental unit was monitored for two weeks to ensure maximum uniformity. Before the start of the experiment, Levene's and F tests were applied to verify the homogeneity of variances in egg production and body weight between treatments. The experimental design was completely randomized, with eight treatments and 11 replicates of three hens each. The lighting program was 16 h of light, using an intensity of 30 lux. The experiment lasted 16 weeks. Temperature and humidity were recorded daily, and their average values for maximum and minimum were approximately 30.67°C, 16.09°C and 70.96%, 36.21%, respectively. The hens were fed 110 g of feed/day and water was provided ad libitum.

2.3. Diets and Treatments

A basal diet (BD) was formulated to meet the nutritional requirements for metabolizable energy, minerals, vitamins, protein, and essential amino acids, except for Trp, which was defined as the first limiting amino acid in dietary profile protein. The contents of metabolizable energy, digestible amino acids, available phosphorus and total calcium of the ingredients, corn, soybean meal and meat meal were determined using near-infrared spectroscopy and used to adjust the experimental diet formulations.

The Trp level of 0.112% g/kg in the diet was obtained without a source of crystalline L-tryptophan (Table 1). The BD was used as treatment 1, with the first level being the Trp - D1 (Table 1). The treatments consisted of eight Trp levels obtained by the supplementation technique (Table 2). The other treatments were obtained with the supplementation of the L-tryptophan biomass 60% in the BD (Table 2). To balance crude protein and energy in the diets, kaolin, L-glutamic acid 98% and starch were used (Table 2). The levels were D1: 0.112%, D2: 0.127%, D3: 0.142%, D4: 0.157%, D5: 0.172%, D6: 0.187%, D7: 0.202%, and D8: 0.225% of digestible Trp (Table 3). Recommendations for nutritional requirements were obtained from [22], as follows: 2.850 kcal/kg of metabolizable energy corrected; 15.76% of crude protein; 0.875% of digestible lysine; 0.858% of digestible methionine+ cysteine; 4.5% of calcium total; 0.332% of availability phosphorus, and other nutrients as presented in Table S1.

Table 1. Feed composition of the basal diet (g/kg).

Ingredients	Basal diet
Grain Corn	715.947
Soybean Meal 46%	121.925
Meat and bone meal 42%	47.413
Fine Limestone	14.578
Coarse Limestone	72.146
NaCl	3.120
L-Met 100%	2.823
L-Lysine 78%	2.713
L-Threonine 98%	1.141
L-Valine 96%	0.922
L-Isoleucine 98%	1.460
L-Arginine 98%	0.458
Choline Chloride 60%	0.710
Lignocellulose	5.064
Premix ¹	1.000
L-glutamic acid 98%	2.518
Starch	1.000
Kaolin	5.063
Total	1000

¹Content per kg of diet: folic acid—1.232 mg; pantothenic acid—26.40 mg; biotin—0.0352 mg; niacin—61.60 mg; vitamin A—4.224 mg; vitamin B1—3.872 mg; vitamin B12—0.02112 mg; vitamin B2—10.032 mg; vitamin B6—5.632 mg; vitamin D3—0.1232 mg; vitamin E—23.584 mg; vitamin K3—3.52 mg; copper—16.00 mg; iron—100.00 mg; iodine—2.40 mg; manganese—140.00 mg; selenium—0.60 mg; zinc—140.00 mg.

Table 2. Composition of experimental diets by supplementation technique (g/kg).

Items (g/kg)	D1	D2	D3	D4	D5	D6	D7	D8
Basal diet	1000	991.439	991.462	991.484	991.505	991.527	991.548	991.582
L-glutamic acid 98%		2.245	1.971	1.698	1.425	1.152	0.879	0.460
Starch		0.980	0.960	0.939	0.919	0.899	0.879	0.848
Kaolin		5.086	5.107	5.129	5.151	5.172	5.194	5.227
L-tryptophan biomass 60%		0.250	0.500	0.750	1.000	1.250	1.500	1.883
Total	1000	1000	1000	1000	1000	1000	1000	1000

D: diet.

Table 3. Treatments, levels and ideal ratio tryptophan ÷ lysine.

Items (%)	1	2	3	4	5	6	7	8
Ideal ratio tryptophan ÷ lysine	5	7	9	1	3	5	7	0
L-tryptophan in diet	.112	.127	.142	.157	.172	.187	.202	.225

D: diet.

2.4. Experimental Procedures and Performance Variables

The body weight was measured at the beginning and end of the assay. Weekly, the leftovers were weighed to quantify feed intake. Egg production and mortality were recorded daily. Egg weight was measured on two consecutive days per week. The variables analyzed were daily feed intake (FI, g/hen d), body weight (BW, g), daily egg production (EP, %), egg weight (EW, g), daily egg mass (EM, g/hen·d), feed conversion ratio by egg mass, corrected for mortality (FCR, g/g), change body weight (cBW, g/hen), Trp intake (I, mg/hen d), tryptophan deposition in egg (D, mg/hen·d), and Trp maintenance (m, mg/hen·d).

The total amount of protein in egg, percentage of Trp of this protein, and total Trp deposited in egg were calculated, using the concentration of protein (6.25×451 mg of nitrogen by g dry matter) and Trp deposited in eggs of hens (108 mg/g nitrogen in egg) obtained from [23]. The dry matter value in the egg was obtained from [24]. The average value of 10.4 mg of Trp for maintenance per metabolic body weight ($\text{kg}^{0.67}$) was obtained by [25,26].

2.5. Egg Assessment and Quality Variables

At the end of the trial two eggs per experimental unit, totaling 22 eggs per treatment, were randomly selected for egg quality analysis. The eggs were evaluated for egg weight, albumen height, yolk weight and eggshell thickness. The variables analyzed were yolk weight (YW, g), eggshell weight (ESW, g), albumen weight (AW, g), eggshell thickness (EST, μm), percentage yolk (PY, %), percentage albumen (PA, %), and percentage shell (PS, %). The eggs were weighed and broken; the yolk was separated from the albumen and shell. The shells were washed and air-dried for 48 hours. They were then weighed, and the thickness was measured. The weight of the albumen was considered as the difference between egg weight and the sum of the weight yolk + shell. The egg weight, yolk weight and eggshell weight were determined using an analytical balance. The thickness was measured with a caliper.

2.6. Cumulative Effect of Dietary Trp on Body Composition

At the end of the trial, a hen with an average BW from each experimental unit was selected for body composition analysis using Dual-energy X-ray

absorptiometry (DXA) technology, totaling 88 hens. The hens were submitted to solid 8-hour fasting for emptying of the digestive tract, then they were weighed for body composition analysis, total mass, area, bone mineral content, bone mineral density, fat mass, and lean using DXA technology. Based on the equations by [27], the body composition in protein, lipid, water, and ash were obtained. The variables analyzed were body water (g/hen), body protein (g/hen), body lipid (g/hen) and body ash (g/hen) of the hens.

2.7. Statistical Analysis and Models to Response Interpretation

The data were tabulated and analyzed for the assumptions of homoscedasticity of variance (Brown-Forsythe) and normality of errors (Cramér–von Mises) using a mixed model. The experimental unit was considered as the random effect and Trp levels as the fixed effect. The variables were subjected to orthogonal contrast analysis for linear and quadratic effects of Trp levels. When an effect was detected, considering a significance at 0.05 ($P \leq 0.05$), regression analysis was applied using the broken-line (BL) models: linear plateau (LP), quadratic-plateau (QP), and linear-linear (LL), according to [28]. The models were adjusted using Trp intake (mg/hen·day). The value in the abscissa corresponding to the first intercept of the quadratic curve of the linear-plateau model was also calculated to determine the ideal Trp intake. The adjusted models were:

$$\text{LP: } \gamma = \phi + \alpha[\lambda - \delta] \quad (1)$$

Where, $(\lambda - \delta)$ is 0 for δ values $> \lambda$, γ is the response, δ is the regressor variable, ϕ is the corresponding response to λ on the orderly axis, λ is the point that indicates a change in the trajectory of γ corresponding to the axis of the abscissas, and α is the slope.

$$\text{QP: } \gamma = \xi + \beta[\tau - \delta]^2 \quad (2)$$

Where, $(\tau - \delta)$ is 0 for δ values $> \tau$, γ is the response, δ is the regressor variable, ξ is the corresponding response to τ on the orderly axis, τ is the point that indicates a change in the trajectory of γ corresponding to the axis of the abscissas, and β is the slope. The value in the abscissa corresponding to the first intercept of the QP curve on the plateau of the LP model (QP₁–LP), was calculated as follows:

$$\text{QP}_1\text{–LP: } \delta = -(-\beta\lambda + \sqrt{\beta\phi - \beta\lambda})/\beta \quad (3)$$

For this broken line two-slope or linear-linear (LL), there was only relation between Trp deposition in egg and Trp intake, according to the following equation:

$$LL: y = \varphi + \alpha[\lambda - \delta] + \beta[\delta - \lambda] \quad (4)$$

Where φ represents the coordinate on the ordinate axis and λ on the abscissa axis of a break point on a curve. α is the slope coefficient of a line when $\delta < \lambda$, and at two-slope model β represents the slope of a line when $\delta > \lambda$. So, by definition $(\lambda - \delta)$ is zero when $\delta > \lambda$ and $(\delta - \lambda)$ is zero when $\delta < \lambda$.

The total or gross efficiency of Trp utilization (kt) was obtained from the ratio of φ to λ for the LP model, and ξ to τ for the QP model, based on parameters fitted to EM, using equations 1 and 2. The kt for QP₁–LP was obtained from the ratio of δ the QP₁–LP and φ the LP model. The efficiency of Trp utilization in egg deposition (ke) was obtained based on equation 4, using the ratio of φ to λ for the LL model. Statistical analyses were performed using SAS 9.4 (Statistical Analysis for Windows, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Performance, Egg Quality and Body Composition

The average values of FI, EP, EW, EM, FCR and FF of hens fed different levels Trp in the diet from 70 to 86 weeks of age are presented in Table 4. According to the probability values for F statistics of ANOVA, linear and quadratic effect (Table 4), Trp levels did not affect FI and EW ($p > 0.05$). EP was affected by the Trp level in the diet and the other variables obtained through the relationship with EP were also affected (Table 4), such as EM, FCR and FF ($p < 0.01$).

The maximum and minimum response values for EP were 94.6% and 81.4%, obtained with 0.187% and 0.112% of Trp in the diet, respectively. Resulting in an amplitude of 13.2% units in EP, which is equivalent to approximately 14% reduction in EP in relation to the maximum EP value, obtained with 0.187% of Trp in the diet (Table 4). This percentage of reduction or modification in the response due to Trp deficiency in the diet remained in the other variables obtained from EP, such as EM, FCR and FE, with 15.0%, 14.5% and 14.5% reduction in relation to the maximum value, respectively. It is

important to highlight the experimental variability, the coefficient of variation (CV) for FI and EW were low, while the CV for EP and other variables obtained using EP were three times the CV for FI and twice that for EW (Table 4).

The average values of YW, ESW, AW, EST, PY, PA and PS of hens fed different levels Trp in the diet from 70 to 86 weeks of age are presented in Table 5. The results obtained for the egg quality variables support the statement that Trp levels did not affect ($p > 0.05$). any of the variables analyzed after 16 weeks of experimentation. The values of CV of the variables were low (Table 5).

Average responses of BW, cBW, and major body chemical components: water, pro-teín, lipid, ash of hens fed different levels of dietary Trp from 70 to 86 weeks of age are presented in Table 6. There was no significant effect ($p > 0.05$) of Trp levels on BW and cBW (Table 6). For the chemical composition of water, protein and ash in the body, a significant effect was detected for the F test ($p < 0.01$) of Trp levels, but it was not possible to model the effect with linear and quadratic adjustment, both were non-significant ($p > 0.05$). The body lipid (γ) content was significant for the F test, linear and quadratic effect (Table 6) of Trp levels (δ) in diet. Considering the highest degree polynomial, that is, quadratic effect ($\gamma = -2930.2\delta^2 + 899.9\delta + 108.83$, $R^2 = 0.3381$), the body lipid presented two minimum points, coinciding with the lowest (0.127% of Trp) and highest (0.225% of Trp) level of Trp in the diet and a maximum point, close to the level of 0.172% of Trp in the diet.

Table 4. Average responses of daily feed intake (FI, g/hen), daily egg production (EP, %), egg weight (EW, g), daily egg mass (EM, g/hen), feed conversion ratio (FCR, g/g) and feed efficiency (FE, g/kg) of hens fed with tryptophan in the diet (TRP, %), from 70 to 86 weeks of age.

Treatments	TRP	FI	EP	EW	EM	FCR	FE
D1	0.112	108.2	81.4	59.2	48.2	2.251	445.1
D2	0.127	107.7	84.6	60.9	51.5	2.093	478.7
D3	0.142	108.4	88.5	60.3	53.3	2.036	491.7
D4	0.157	108.9	92.8	60.1	55.8	1.954	512.3
D5	0.172	108.7	94.1	59.8	56.3	1.932	518.5
D6	0.187	109.0	94.6	60.0	56.7	1.924	520.3
D7	0.202	107.7	93.8	59.4	55.7	1.937	516.8
D8	0.225	108.0	93.1	59.3	55.2	1.957	511.3
Average		108.4	90.6	59.8	54.2	2.008	500.0
SEM		0.2	0.5	0.2	0.3	0.012	3.0
CV, %		1.9	5.8	3.3	6.1	6.1	5.8
P-value							
ANOVA		0.329	<.0001	0.507	<.0001	<.0001	<.0001
Linear effect		0.949	<.0001	0.307	<.0001	<.0001	<.0001
Quadratic effect		0.108	<.0001	0.224	<.0001	<.0001	<.0001

D: diet. SEM: Std Error of mean. CV: Coefficient of Variation.

Table 5. Average of yolk weight (YW, g), eggshell weight (ESW, μ m), albumen weight (AW, g), eggshell thickness (EST, μ m)), percentage yolk (PY, %), percentage albumen (PA, %), and percentage shell (PS, %) of hens fed with tryptophan in the diet (TRP, %), from 70 to 86 weeks of age.

Treatments	TRP	YW	ESW	AW	EST	PY	PA	PS
D1	0.112	15.4	5.9	37.8	400.9	26.0	64.0	9.9
D2	0.127	15.7	6.2	39.0	404.8	25.7	64.1	10.2
D3	0.142	15.4	6.1	38.9	407.6	25.5	64.4	10.1
D4	0.157	15.5	6.1	38.5	407.4	25.8	64.0	10.1
D5	0.172	15.5	5.9	38.4	398.9	25.9	64.2	9.9
D6	0.187	15.2	6.0	38.7	400.7	25.4	64.6	10.0
D7	0.202	15.3	6.0	38.2	401.1	25.8	64.3	10.0
D8	0.225	15.4	5.9	38.1	401.2	26.0	64.1	10.0
Average		15.4	6.0	38.4	403.1	25.7	64.2	10.0
SEM		0.1	0.03	0.2	1.2	0.1	0.1	0.04
CV, %		3.8	4.5	4.3	3.0	3.4	1.6	3.8
P-value								
ANOVA		0.595	0.138	0.772	0.635	0.693	0.925	0.798
Linear effect		0.342	0.247	0.605	0.349	0.956	0.547	0.640
Quadratic effect		0.955	0.124	0.213	0.563	0.220	0.463	0.455

D: diet. SEM: Std Error of mean. CV: Coefficient of variation.

Table 6. Average responses of body weight (BW, g/hen), change body weight (cBW, g/hen), body water (Water, g/hen), body protein (Protein, g/hen), body lipid (Lipid, g/hen) and body ash (Ash, g/hen) of hens fed with tryptophan in the diet (TRP, %), from 70 to 86 weeks of age.

Treatments	TRP	BW	cBW	Water	Protein	Lipid	Ash
D1	0.112	1,810	155.0	913.5	274.3	177.0	56.6
D2	0.127	1,808	155.0	870.4	261.1	165.3	55.1
D3	0.142	1,838	149.0	879.7	261.3	179.1	54.5
D4	0.157	1,817	143.0	980.8	291.4	184.5	60.6
D5	0.172	1,852	150.0	906.9	269.4	184.6	56.1
D6	0.187	1,856	144.0	896.4	268.9	166.2	56.7
D7	0.202	1,820	87.0	915.7	272.0	166.7	56.7
D8	0.225	1,795	129.0	902.3	268.0	166.3	55.8
Average		1,830	136.0	912.9	272.1	173.7	56.8
SEM		9.0	8.0	5.5	1.6	1.1	0.3
CV, %		5.0	5.9	6.0	5.9	6.0	5.8
P-value							
ANOVA		0.474	0.476	<.0001	<.0001	<.0001	<.0001
Linear effect		0.965	0.100	0.528	0.879	0.0007	0.729
Quadratic effect		0.104	0.911	0.130	0.211	<.0001	0.097

D: diet. SEM: Std Error of mean. CV: Coefficient of variation.

3.2. Models for Response Interpretation to Estimate Dietary Tryptophan Requirement

According to Table 7, both the LP and QP models estimate a maximum response of 94.4% for EP. The LP model identifies a breakpoint at 180.9 mg/hen, while the QP model estimates it at 207.3 mg/hen. The R^2 values for both the LP and QP models were similar, at 78% (Table 7). A distinction between the models was observed only in the model selection statistic, AIC, where the LP model had the lower value (343.3) compared to the QP model (347.6). The QP_1-LP occurs at 186.2 mg/hen, corresponding to 89% of the QP model's estimated breakpoint (Table 7).

Table 7. Estimated parameters of the linear-plateau (LP) and quadratic-plateau (QP) models of the variables of daily egg production, daily egg mass, feed conversion ratio and feed efficiency relationship between daily tryptophan intake of hens from 70 to 86 weeks of age.

Variables (y)	Linear plateau (LP)					Quadratic plateau (QP)					¹ QP ₁ -LP
	ϕ	β	λ	R ²	AIC	ξ	β	τ	R ²	AIC	
Daily egg production, %	94.4	-0.22	180.9	78.0	343.3	94.4	-0.002	207.3	78.0	347.6	186.2
Daily egg mass, g/day	56.1	-0.18	168.4	78.3	267.0	56.2	-0.002	188.9	79.0	269.1	174.7
Feed conversion ratio, g/g	1.921	0.006	182.1	81.1	-194.3	1.923	0.00005	203.0	80.9	-192.7	182.7
Feed efficiency, g/kg	520.2	-1.26	182.5	80.2	562.6	520.2	-0.011	206.3	80.0	566.6	182.5

y: response variable; δ : daily tryptophan intake (mg/hen); ϕ : is maximum response; β : is the slope; λ : is requirement for maximum response (mg/hen); R²: Coefficient of determination; AIC: Akaike information criterion; ξ : is maximum response; τ : is requirement for maximum response (mg/hen); ¹QP₁-LP, 1st interception of QP on the plateau of the LP.

The estimate maximum response was 56.1, 1.921, and 520.2 for EM, FCR, and FE using LP, and 56.2, 1.923, and 520.2 for EM, FCR, and FE using QP, respectively. The co-efficient of determination (R^2) indicated similar goodness of fit between the LP and QP models for each response variable (Table 7). Differences in R^2 values between models, LP and QP, were minimal, with variations of 0.7, 0.2, and 0.2 for EM, FCR, and FE, respectively. However, model selection based on the Akaike information criterion (AIC) favored the LP model, which consistently exhibited lower AIC values across all variables. The relationship between the Trp recommendations in the diet estimated by LP was close to 89% of the value found for QP. Meanwhile, the QP_1 –LP was 90%, therefore very close to the value estimated by LP (Table 7). Based on the dose–response analysis, the Trp level of 186.2 mg/hen, as estimated by QP_1 –LP to EP, can be recommended as it meets the requirements for the remaining response variables (Table 7).

3.3. Efficiency of tryptophan utilization for egg deposition

Based on the adjusted parameters for EM estimated using the LP, QP, and the QP_1 –LP, as presented in Table 7, the values of k_t were calculated. The estimated k_t values were 0.63 or 63% for the LP model, 0.56 or 56% for the QP model, and 0.61 or 61% for the QP_1 –LP model. The average values of Trp Intake, Trp deposited in egg, and Trp used to maintenance of hens fed different levels Trp in the diet from 70 to 86 weeks of age are presented in Table 8. According to the probability values for F statistics of ANOVA, linear and quadratic effect (Table 8), Trp levels did not affect the Trp used to maintenance the BW of the hens ($p > 0.05$). The average amount of tryptophan allocated for maintenance was 15.6 mg/hen day, regardless of dietary Trp concentration.

The amount of Trp required per gram of egg mass, expressed in mg of Trp per g of egg was calculate and values were 3.0 and 2.71 for the LP model, 3.36 and 3.07 for the QP model, and for QP_1 –LP were 3.11 and 2.82, respectively, to total and net utilization the amount of Trp required per gram of egg mass.

Table 8. Partitioning of tryptophan intake to maintenance and egg deposition according to tryptophan in the diet (TRP, %), of hens fed from 70 to 86 weeks of age.

Treatments	TRP	Tryptophan Intake	Tryptophan Deposited in Egg	Tryptophan Maintenance
D1	0.112	121.2	91.0	15.5
D2	0.127	136.7	97.4	15.5
D3	0.142	153.9	100.7	15.6
D4	0.157	171.0	105.4	15.5
D5	0.172	186.9	106.5	15.7
D6	0.187	203.9	107.2	15.7
D7	0.202	217.6	105.2	15.5
D8	0.225	243.0	104.3	15.4
Average		180.2	102.398	15.6
SEM		3.7	0.63	0.1
CV, %		20.6	6.1	3.4
<i>P</i> -valor				
ANOVA		<.0001	<.0001	0.473
Linear effect		<.0001	<.0001	0.944
Quadratic effect		0.106	<.0001	0.103

D: diet. SEM: Std Error of mean. CV: Coefficient of Variation.

The variables Trp intake and Trp deposited in the egg were affected by Trp levels, showing linear and quadratic effects ($p < 0.05$). The maximum and minimum response values for Trp deposited in the egg were 107.2 mg/hen and 91.0 mg/hen, obtained with 0.187% and 0.112% of Trp in the diet, respectively. Resulting in an amplitude of 16.2 mg/hen the Trp deposited in the egg, which is equivalent to approximately 15.1% reduction in Trp deposited in the egg in relation to the maximum value obtained with 0.187% of Trp in the diet (Table 8).

The relationship between Trp intake and Trp deposited in egg of hens was adjusted by equation: $y = 107.6 - 0.263[187 - \delta] - 0.04[\delta - 187]$, $R^2 = 95\%$, Figure 1. Based on model LL, the Trp intake of 187 mg/hen day, as estimated to maximize Trp deposited in egg, can be recommended as it meets the requirements for the remaining response variables (Figure 1), including EP. The k_t was obtained using the ratio of $\phi = 107.6$ mg/hen to $\lambda = 187$ mg/hen, resulting in 0.575 or 57.5%. Taking the reciprocal the k_t , $1/k_t$, the value obtained was 1.74 mg Trp/g egg mass. The value of k_e and $1/k_e$ were obtained based on considering only partitioning to Trp deposited in the egg, therefore of Trp intake used to maintenance was subtracted (Trp intake for deposition is 171.4 mg/hen). The value of k_e was 0.627 or 62.7%, and $1/k_e$ was 1.59 mg of Trp per g of Trp deposited.

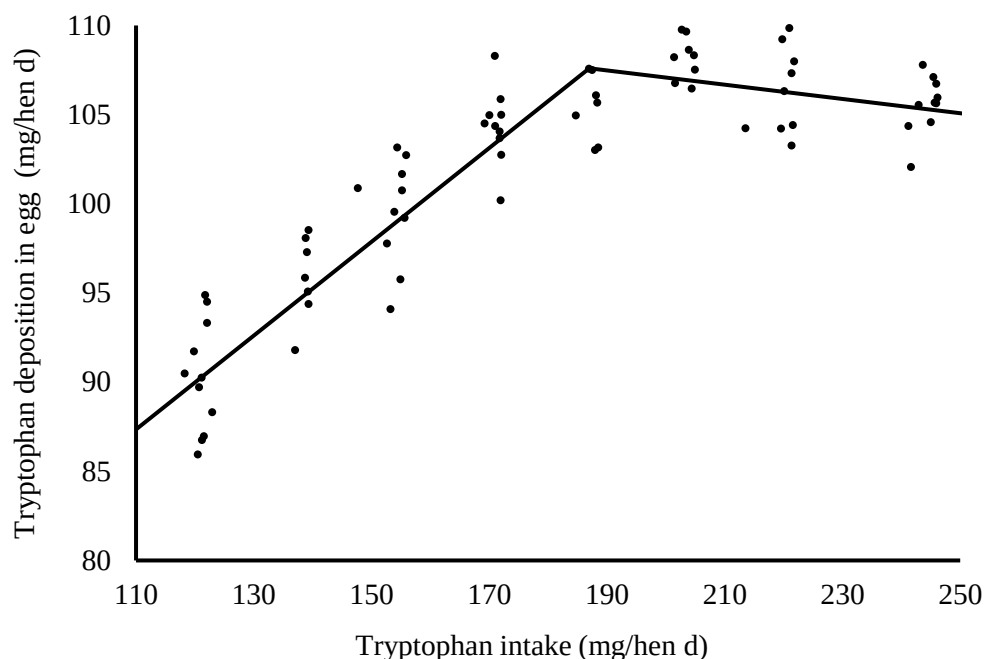


Figure 1. Relationship between tryptophan intake and tryptophan deposition in egg of hens from 70 to 86 weeks of age. Adjusted equation: $y = 107.6 - 0.263[187 - \delta] - 0.04[\delta - 187]$, $R^2 = 95\%$.

4. Discussion

4.1. Responses of Brown Laying Hens to Dietary Trp

Due to the limited number of studies involving brown egg laying hens, this section includes relevant research conducted with both white and brown Leghorn strains. Absence of effect of Trp on feed intake and egg weight has also been reported in previous studies [29,30], who evaluated Trp levels in hen diets and did not observe a significant effect on feed intake (Table 4). [31] reported that egg weight could be related to the inadequate amount of one or more other amino acids, but since the amino acids in the diet were within the recommended levels for laying hens, including Trp at different levels, such differences may not have been found [32,33].

Daily egg production followed a linear or quadratic plateau pattern (Table 7), consistent with the findings in the literature [33-35]. Although the precise mechanisms through which tryptophan affects egg production remain unclear, it is important to note that tryptophan is a precursor in the synthesis of melatonin. Melatonin has been shown to play a crucial role in ovarian cell regeneration,

promoting follicular development and ovulation, which may enhance egg production in laying hens [36].

Following the same trend, egg mass, feed conversion ratio, and feed efficiency showed a linear and quadratic effect according to the dietary levels of Trp (Table 4), with the highest mass obtained from 175 mg/hen d (Table 7). The adequate egg mass was achieved Trp intake from 165 mg/hen d, in hens aged 53 to 58 weeks [37]. Similar observations have been reported regarding egg mass and feed conversion ratio in previous studies [32,33].

No significant effects of dietary Trp levels were observed on egg quality parameters in the present study (Table 5). [38] reported that adequate Trp intake is essential for follicular development, which may be associated with changes in yolk proportion. However, the absence of differences in yolk weight among treatments suggests that even the lowest dietary tryptophan levels were sufficient to support this physiological requirement. Consistent with our findings, previous studies also reported no significant effects of varying tryptophan concentrations on yolk weight [33,39,40]. Similarly,[41] found no significant influence of dietary tryptophan on eggshell thickness or albumen weight. As noted earlier, melatonin, a metabolite derived from tryptophan, has been shown to enhance nutrient absorption, including calcium (Ca^{2+}), thereby potentially improving eggshell quality [42]. Nevertheless, the mechanisms by which dietary Trp modulate calcium metabolism, transport, bone integrity, and eggshell formation remain largely unexplored and warrant further investigation.

The egg is composed primarily of yolk, albumen, and eggshell with membrane, which represent approximately 30%, 60%, and 10% of total egg weight, respectively [43]. In the present study, dietary Trp levels did not influence the relative proportions of yolk, albumen, or eggshell (Table 5).

A positive linear effect was observed for Trp intake as dietary tryptophan levels increased (Table 8). The partitioning of Trp toward maintenance requirements was also influenced by dietary Trp levels (Table 8). This outcome was expected, as maintenance requirements are closely associated with the hens' metabolic weight, which is calculated as body weight raised to 0.67 power. Notably, body weight itself was not affected by dietary Trp levels (Table 6). The Trp deposition exhibited both linear and quadratic responses (Table 8), with a maximum deposition of 107.6 mg/hen d, observed at a dietary intake of

187 mg/hen d (Figure 1). This response pattern reflects an increase in Trp deposition as dietary levels approach the hens' physiological requirement, followed by a decline when intake exceeds this threshold [44,45].

4.2. Efficiency Utilization of Trp for Egg Production

This research was conducted to evaluate the efficiency of Trp utilization and determine the ideal Trp intake for aged brown layers using a L-tryptophan biomass 60% source. Based on the results obtained, it was possible to extract values of Trp utilization efficiency for aged brown layers and derive nutritional recommendations for Trp in the diets of these hens.

The values of k_t and k_e were determined to be 61% and 63%, respectively. These indices refer to the efficiency with which the aged layers utilized Trp, denotes the fraction used for maintenance and egg production (k_t), whereas, k_e represents the fraction of ingested Trp destined for protein deposition in the egg. These results support the hypothesis formulated in this research that commercial layers are more efficient than heavy type breeders [20,46,47].

Therefore, the higher value of utilization efficiency observed in commercial layers is supported by the length of the laying sequence of these birds, since heavy type breeders have shorter laying sequences [21] and longer pauses [21] between sequences compared with commercial layers. This genetic condition favors greater utilization for egg production, especially at the 94.6% egg production level observed in this study.

Despite this confirmation, the k_e value for tryptophan was lower than for other amino acids, such as lysine (85%–87%), methionine + cysteine (76%), and threonine (75%), as reported by [48,49]. This finding can be explained in two ways. The first is strictly related to Trp metabolism as a multifunctional molecule essential for physiological processes, from X action to egg protein deposition. This partitioning beyond egg production means that Trp is not recovered in the egg and, for this reason, k_e is low, but Trp is vital for maintaining the health and well-being of the bird [50]. The second explanation relates to the methodology applied, especially the degree of Trp deficiency applied in DB. The latest studies on Trp for layers used the supplementation technique and commonly used close to a 10% deficiency of Trp in the diet

[18,19]. A low degree of deficiency may not be sufficient to limit the protein synthesis destined for egg deposition, especially for layers, which mobilize body reserves to sustain egg production [20,46-48]. The trait of preserving reproduction obscures, attenuates, and directly influences the measurement of k_e by conventional methods.

In this research the degree of deficiency employed (15%) was sufficient only to produce an effect on egg production after 16 weeks of experimentation, among the directly measured variables such as feed intake and egg weight. Therefore, it is important to note that aged brown or semi-heavy layers have body reserves, as indicated by the cBW responses (Table 6). The capacity for body reserve was analyzed considering its influence on the adaptation period of the requirement study [51]. Those authors used laying quails, an animal model with low body reserve capacity, and used a diet with a 390% deficiency of the first limiting amino acid; at the end of the study, the authors recommended a total of 28 days as the period sufficient to impose the deficiency on the birds and derive results. Finally, the degree of deficiency of the limiting amino acid and the physiological state of the birds should be considered to establish dietary levels and the experimental period, in order to obtain clear responses for feed intake, egg weight, and egg quality, which were not observed in this research.

In addition, the study by [17] imposed a 32% deficiency in commercial layers at the beginning of the laying peak (31–37 weeks) with a 42 days experimental period, and obtained clear responses for feed intake, production, and egg weight.

The results obtained by [17] showed that the birds mobilized body reserves to the point of recording negative cBW values, demonstrating that the physiological status of young birds is greatly influenced by the effects of dietary deficiency.

From the results of [17], a k_e value of 58.6% was derived. Based on this value, it is apparent that the birds in the present study were more efficient in the use of dietary Trp. The higher efficiency observed in this study is related to the potential for egg production: the maximum egg production achieved here was 94.6%, whereas in the study of [17] it did not exceed 85%. Despite the difference in age of the birds between 70-86 weeks (present study) *versus* 31–37 weeks [17], in this study the birds exhibited longer laying sequences and

shorter pauses between laying sequences. This underscores the importance of constantly reevaluating nutritional requirements, given the improvements in the genetic progress of modern birds.

4.3. Nutritional constant for egg mass or mg tryptophan per g egg mass, $1/k_t$ and $1/k_e$

Traditionally, amino acid requirements for laying hens have been estimated using linear regression of empirical data or by developing partitioning models based on assumed linear relationships between nutrient intake and output [25,52,53]. The estimation of Trp requirements per unit of egg output, expressed as mg Trp per g egg mass was based on this linear assumption. Different response functions (linear and non-linear) were exhaustively evaluated [45]. It was observed that the point marking the end of the linear response phase represented a region where all models converged toward the mean estimated requirement [45]. Beyond this point, divergence among functions became more evident, depending on the specific parameterization of the asymptotic response [45].

Comparison based on $1/k_e$ values eliminates the confounding effect of maintenance requirements on the utilization of Trp for egg mass production. The estimates derived from the combination of the QP₁–LP and LL models were 2.82 and 1.54 mg/g, respectively. Although obtained through different modeling approaches, these values exhibit comparable predictive capacity, as both are grounded in the same efficiency of utilization. The key distinction lies in the input variable: QP₁–LP uses grams of egg mass, a direct measurable output, whereas LL employs the amount of tryptophan deposited (mg). Previous studies have typically used egg mass as the input variable [25,52,54].

Pioneering studies reported a requirement of 2.25 mg of Trp per gram of egg mass [25,54]. Unlike the present study, those authors estimated Trp requirements for both egg mass production (2.25 mg/g) and body weight maintenance (10.25 mg/kg) simultaneously, which introduced a correlation between input variables—egg mass and body weight. This correlation influenced the intersection point of the response curves and, consequently, the partitioning estimate for maintenance. In the present study, the maintenance requirement was fixed based on literature values (10.4 mg/kg^{0.67}) and

subtracted from total intake to isolate the Trp requirement for egg mass production: $[(174 \text{ mg} - 16.4 \text{ mg}) \div 56.1 \text{ g}]$.

To reduce the correlation between input variables and improve the reliability of estimates, some studies have employed datasets compiled from multiple experiments. Using this approach, [52] reported a requirement of 2.62 mg Trp/g of egg mass. In the current study, the net Trp deposition per gram of egg mass was estimated at 1.89 mg/g. Therefore, k_e can be calculated as the ratio of deposition to intake. Based on this approach, k_e values extracted from [25,52] were 84% and 71%, respectively.

The differences in k_e between these studies and the current work are likely attributable to differences in hen strain. The present study used brown-egg layers, which are less efficient than the light hybrids used in previous studies. The $1/k_e$ values determined here can be applied in factorial models to support nutritional recommendations beyond the specific experimental conditions of this trial.

4.4. Nutritional Recommendations for Trp in the Diet

A secondary objective of this research was to determine the ideal intake of Trp for aged brown laying hens using the L-tryptophan biomass 60% source. The methodology applied in this research, considering the formulation of experimental levels and the experimental period, was sufficient to modify the hens responses, allowing productive responses to be obtained (Table 4), which supported the derivation of nutritional recommendations for Trp in the diet for these birds. Based on the results obtained, the ideal Trp intake is 187 mg/hen per day. This intake was defined following the highest value criterion to meet the requirements of other variables, especially egg production, which was estimated at 186.2 mg/hen per day, close to the value obtained from the relationship between deposition and Trp intake (Figure 1).

The responses obtained from the increasing levels of Trp in the diet allowed mathematical functions (linear-plateau, quadratic-plateau, and linear-linear) to be fitted, which were used to interpret the responses and extract nutritional recommendations based on the parameters of these functions. The parameters λ and τ of the functions represent the change point in the response corresponding to the Trp intake axis, therefore, it is taken as the ideal Trp

intake. The τ values were on average 13% higher compared to λ values (Table 7), for this reason, an intermediate point of Trp intake between λ and τ was calculated, using the ϕ value from the linear-plateau model in the quadratic curve equation (Eq. 3). The Trp intake values obtained with this procedure (186.2, 174.7, 182.7, and 182.5 mg/hen/day for egg production, egg mass, feed conversion, and feed efficiency, respectively, Table 7) were close to the estimate obtained from the relationship between deposition and Trp intake of 187 mg/hen/day, thus providing a robust recommendation obtained by applying several statistical tools [55].

The recommendation proposed by [18] was approximately 33% higher than the value defined as the recommendation in this research. The difference between the recommendations is related to the utilization efficiency, which in this study was 63%, while the efficiency value of [18] was 46%; therefore, to produce 53 g/day, of the laying hens from [18] required an intake of 249 mg Trp/hen/day.

A recent study conducted with Hy-Line Brown laying hens, from 70 to 74 weeks of age, recommended a Trp intake between 209 and 225 mg/hen per day to produce between 47.5 and 48.7 g/day of egg mass, resulting from the relationship between production (72% and 75%) and egg weight (65 g and 64 g), respectively, measured over a four-week period [19]. From the utilization efficiency values extracted from [19], Hy-Line Brown hens at 70 weeks of age showed efficiency between 46% and 44%, respectively, considering a partition of 15.5 mg of Trp for maintenance. The better efficiency presented in this research is related to the genetic potential for egg production; the maximum egg production was 94.6%, while in the study by [19] it did not exceed 75%. Despite the similarity in hen age, the degree of deficiency imposed by the formulation of the experimental levels and the 16-week experimental period provided better conditions to establish the effects of dietary Trp levels and consequently extract the dietary Trp recommendation. This demonstrates the relevance of the method applied for nutritional requirement studies in poultry. For the same reason, that is, the difference in productive potential, the Trp intake recommendation in this research was higher than the 171 mg/hen per day found by [17], showing the need to update the Trp requirement for brown layers.

The recommendation established in the present study (187 mg/hen per day) is based on an average bird with a body weight of 1.830 kg and a daily egg mass production of 56.7 g/hen. This recommendation was lower than that presented by [22] of 201 mg/hen per day and close to the updated recommendation in 2024 of 187 mg/hen per day, considering the average described in this research. The systematic review conducted by [56] resulted in an average of 167 mg/hen per day, obtained from eight publications that used Leghorn, white, or brown hens.

According to [57], the effect of Trp in the diet is greater during the latter part of the laying period, attributing to Trp the effect of attenuating the decline in egg production due to aging. In this sense, there is a hypothesis based on the relationship between Trp and estradiol, in which estradiol increases after the first egg and continues to increase with the hen's age [58], and its relationship with plasma progesterone has a negative correlation with egg production [59]. Based on this, the hypothesis attributes to the higher estrogen level a causal, but not exclusive, factor for the decrease in egg production in aged layers [60]. The improvement in egg production in aged laying hens may be associated with a decrease in estrogenic activity or simply with attenuating the deleterious effects on Trp metabolism, mediated by increasing Trp levels in the plasma, supplied in the hens' diet. Although not determined in this research, it is known that estrogens can interfere with the activity of enzymes involved in tryptophan metabolism, such as tryptophan oxygenase and kynureninase [61-63].

4.5. L-Tryptophan Biomass 60%

This research evaluated a new Trp source, L-tryptophan biomass 60%, complementing the digestibility studies [5] and bioavailability studies [6]. Requirement studies assist in the development of new amino acid sources due to the methodology employed, which uses higher doses of the Trp source in the diet, a longer experimental period, and different ways of interpreting the animal response. As seen in the results of this research, there was no evidence requiring concern that could be attributed to the new Trp source. Therefore, the reassessment of Trp requirements for brown layers allowed for an update in the nutritional recommendation, in addition to evaluating any adjuvant effect of biomass on the supplemental L-tryptophan in the diet. The L-tryptophan

biomass 60% is obtained through a fermentation process and after a steam heating step, granules are formed; therefore, Trp is in granular form. The biomass, which corresponds to approximately 40%, contains protein, essential amino acids, organic acids, and carbohydrates [64], which by itself justifies conducting trials to precisely measure its effect in poultry diets.

The results in this research support the analysis of some hypotheses related to the contribution of biomass on amino acid utilization for egg production. The first hypothesis that can be evaluated regarding L-tryptophan biomass 60% concerns the presence of some compound that may have an action or act as an antinutritional factor. Analyzing the laying hens responses, there was no effect from the treatments, especially for feed intake and egg weight, which were statistically equal, ruling out any negative effect from increasing the inclusion of the L-tryptophan biomass 60% source on the laying hens responses.

The second hypothesis concerns the presence of some factor in the biomass that could positively contribute to improving the laying hens' responses. A precise analysis rules out the possibility of mobilizing body reserves to maintain egg production since there was an improvement in production with positive responses for cBW, without association with Trp levels in the diet.

The third hypothesis refers to digestibility for laying hens. In this research, it was considered that 100% of L-tryptophan biomass 60% was digestible. Although Trp excretion was not measured, it is expected that it was not affected. This hypothesis is supported by the birds' responses, considering that at the highest levels of Trp there was no reduction in performance responses (Table 4) and egg quality (Table 5). On the other hand, the granular physical form of L-tryptophan biomass 60% may be a positive characteristic considering that the granule may slow down the transit rate and improve the absorption, transport, and utilization of Trp. [65] observed that for each additional gram of free amino acids per kg of feed, egg production and egg mass decreased by 3 2.5 g per day, respectively. This means that free 'synthetic' amino acids are not as well utilized as protein-bound amino acids when birds are fed a restricted amount of feed. Although not measured, future studies may evaluate passage rate and confirm whether the granular physical

form of L-tryptophan biomass 60% has the capacity to influence the passage dynamics in the gastrointestinal tract.

5. Conclusions

This study determined that the optimal tryptophan intake for aged brown laying hens is 187 mg/hen per day, with high utilization efficiency (63%). Supplementation with L-tryptophan biomass 60% was effective, safe, and had no negative effects on performance or egg quality. These findings update Trp nutritional recommendations and show that hens at the end of the laying cycle can maintain high productive efficiency.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Table S1: Nutrient composition.

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Abbreviations

The following abbreviations are used in this manuscript:

5-HT	Serotonin
AIC	Akaike information criterion
AW	Albumen weight
BD	Basal diet
BL	Broken-line
BW	Body weight
cBW	Change body weight
CV	Coefficient of variation
D	Tryptophan deposition in egg
DAHPSynthase	3-deoxy-D-arabino-heptulosonate-7-phosphate synthase
DEXA	Dual-energy X-ray absorptiometry
E4P	Erythrose-4-phosphate
EM	Egg mass
EP	Egg production
EST	Eggshell thickness
ESW	Eggshell weight
EW	Egg weight
FCR	Corrected for mortality
FI	Feed intake
I	Trp intake
IPA	Indole-3-pyruvic acid
k	Trp utilization efficiency
k _e	Trp utilization in egg deposition
k _t	Efficiency of Trp utilization
LL	Linear- linear
LP	Linear-plateau
m	Trp maintenance
PA	Percentage albumen
PEP	Phosphoenol-pyruvate
PS	Percentage shell
PY	Percentage yolk
QP	Quadratic-plateau
R ²	Coefficient of determination
Trp	Tryptophan
YW	Yolk weight

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Table S1. Nutrient composition calculated.

Nutrients	Basal diet
Dry matter, %	88.836
Crude fiber, %	2.198
Crude protein, %	14.560
Metabolizable energy correct, kcal/kg	2,825
Digestible Lysine, %	0.746
Digestible Methionine, %	0.487
Digestible Methionine + Cysteine, %	0.683
Digestible Threonine, %	0.525
Digestible Tryptophan, %	0.112
Digestible Arginine, %	0.777
Digestible Glycine + Serine, %	1.255
Digestible Valine, %	0.651
Digestible Isoleucine, %	0.599
Digestible Leucine, %	1.151
Digestible Histidine, %	0.322
Digestible Phenylalanine, %	0.577
Digestible Phenylalanine + Tyrosine, %	1.018
Potassium, %	0.478
Sodium, %	0.160
Chlorine, %	0.354
Total calcium, %	4.000
Available Phosphorus, %	0.345
Total Choline, mg/kg	1,186
Linoleic Acid, %	1.473

CAPÍTULO 4. IMPLICAÇÕES

A biodisponibilidade e a exigência de L-triptofano são fundamentais para o desempenho e a saúde das poedeiras. Além disso, sua biodisponibilidade impacta a saúde intestinal e óssea das aves, podendo contribuir para a melhora da qualidade da casca dos ovos e auxiliar a resposta ao estresse, fatores que são críticos para manter a longevidade e a produtividade.

Sabendo que a biodisponibilidade abrange os processos de digestão, absorção, metabolismo e utilização dos nutrientes, o capítulo 2 se baseia na metodologia empregada por Littell (1997). Neste estudo, foram comparadas duas fontes cristalinas de L-triptofano, com concentrações de 60% e 98%, respectivamente. A biodisponibilidade do L-triptofano 60% granulado foi considerada equivalente a 100%, o que indica que essa fonte apresenta a mesma bioequivalência que a fonte cristalina de L-triptofano 98%. Em termos práticos, isso significa que ambas as fontes fornecem a mesma quantidade de L-triptofano disponível para absorção e utilização no organismo.

O capítulo 3, foi utilizado para determinar a exigência do L-triptofano 60%. Os resultados da relação ideal do aminoácido, teve como princípio a eficiência de utilização, com isso, foi possível estimar a relação ideal do L-triptofano 60%.

O estudo utilizou o modelo Broken-line para determinar a exigência ideal de L-triptofano 60% nas dietas das poedeiras. Isso proporciona uma base sólida para ajustes precisos na formulação de rações, maximizando a eficiência de utilização do aminoácido e promovendo um melhor desempenho produtivo.