**GABRIEL DA SILVA VIANA**

# RESPONSES TO REDUCTION ON DIETARY CRUDE PROTEIN AND SUPPLEMENTATION OF NON-ESSENTIAL NITROGEN; DIETARY ESSENTIAL TO NON-ESSENTIAL NITROGEN OPTIMUM RATIO FOR WHITE COMMERCIAL LAYERS

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Zootecnia, para obtenção do título de *Doctor Scientiae*.

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A Deus pelo dom da vida e pela força para lutar por meus objetivos.

Aos meus pais Ávila e Valéria pelo amor incondicional, carinho, amizade e paciência.

Às minhas irmãs Maria Luisa e Mariana pelo amor, amizade e convivência

**DEDICO.**

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# BIOGRAFIA

Gabriel da Silva Viana, filho de Ávila Viana Filho e Valéria Manhães da Silva, nasceu em 24 de maio de 1989, em Macaé - RJ.

Em 2007 iniciou o Curso de Zootecnia pela Universidade Federal de Viçosa, colando grau em 27 de janeiro de 2012.

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# SUMÁRIO

# RESUMO

VIANA, Gabriel da Silva, D.Sc., Universidade Federal de Viçosa, março de 2017. **Redução da proteína bruta em dietas suplementadas ou não com nitrogênio não essencial e relações nitrogênio essencial:nitrogênio não essencial para galinhas poedeiras leves.** Orientador: Melissa Izabel Hannas. Coorientadores: Sérgio Luiz de Toledo Barreto e Luiz Fernando Teixeira Albino.

Foram conduzidos dois ensaios experimentais com objetivo de avaliar o efeito da redução da proteína bruta em dietas, suplementadas ou não com nitrogênio não essencial (experimento I), e de diferentes relações dietéticas de nitrogênio essencial:nitrogênio não essencial (experimento II) para poedeiras leves. Em ambos ensaios, o período experimental teve duração de 112 dias, sendo subdividido em 4 períodos de coleta de dados de 28 dias cada. No experimento I, 240 galinhas poedeiras Hy-Line W-36 aletoriamente distribuídas a 40 unidades experimentais, divididas em cinco grupos de tratamentos com 48 aves cada. Os tratamentos consistiram de 3 rações com os níveis de proteína bruta de 170.0, 150.0 e 130.0g de proteína/kg de dieta com as relações nitrogênio essencial:nitrogênio não essencial de 37/63, 42/58 e 47/53, respectivamente. Adicionalmente, as duas rações contendo os níveis de proteína bruta de 150.0 e 130.0g de proteína/kg de dieta, foram suplementadas com 37.60 e 60.80g de ácido glutâmico/kg de dieta, respectivamente, com objetivo de igualar a relação nitrogênio essencial:nitrogênio não essencial da dieta de maior nível proteico (37/63). A redução dietética da proteína bruta de 170.0g/kg para 150.0g/kg resultou na redução do peso e massa de ovos, enquanto a redução para 130.0g/kg além de acarretar piora das variáveis antes descritas, também piorou a conversão alimentar por massa de ovos, reduziu o consumo de ração e o peso de albumen. Não observou-se diferenças no desempenho e qualidade de ovos de aves alimentadas com dietas em que o conteúdo de proteína foi reduzido. A suplementação de ácido glutâmico em dietas contendo 150.0g de proteína/kg promoveu mesmo desempenho das aves alimentadas com 170.g de proteína/kg, enquanto em dietas contendo 130.0g de proteína/kg a inclusão de ácido glutâmico resultou nas piores médias de desempenho e qualidade de ovos em comparação aos demais tratamentos. Os resultados demonstram que o déficit de nitrogênio não essencial resulta em piora no desempenho de galinhas poedeiras e revela potencial de utilização de ácido glutâmico como fonte de nitrogênio não essencial, desde que utilizado em baixas concentrações. No experimento II, 360 galinhas Hy-Line W-36 foram distribuídas em seis tratamentos, com dez repetições e seis aves por unidade experimental. Os tratamentos foram obtidos a partir da suplementação gradativa da mistura de L-alanina, L-glicina e ácido glutâmico (proporção 60%/20%/20%) em uma dieta basal com reduzida proteína bruta e relação nitrogênio essencial:nitrogênio não essencial de 55/45 com objetivo de produzir as relações entre nitrogênios de 55/45, 52/48, 48/52, 44/56 e 41/59. Uma sexta dieta com maior teor de proteína bruta, denominada grupo controle, foi formulada com relação nitrogênio essencial:nitrogênio total de 41/59. As relações de 55/45 e 52/48 resultaram em menor peso de ovo e pior conversão alimentar por massa de ovos em comparação ao grupo controle. Observou-se redução nos valores de massa de ovos somente no grupo de aves alimentado com a relação de 55/45. Baseado nos resultados, recomenda-se que a relação dietética de nitrogênio essencial:nitrogênio não essencial para poedeiras leves não ultrapasse o valor de 48/52.

# ABSTRACT

VIANA, Gabriel da Silva, D.Sc., Universidade Federal de Viçosa, July, 2013. **Laying hen responses to reduction on dietary crude protein and non-essential nitrogen supplementation; dietary essential to non-essential nitrogen optimum ratio for white commercial layers.** Adviser: Melissa Izabel Hannas. Co-Advisers: Sérgio Luiz de Toledo Barreto and Luiz Fernando Teixeira Albino.

Two trials were performed in order to evaluate laying hen responses to reduction on dietary crude protein supply and non-essential nitrogen supplementation (Experiment I) and to determine the essential to non-essential nitrogen ratio, which warrants optimum performance, and egg quality of laying hens (Experiment II). Both trials lasted 112 days, being divided in four 28-day intervals. In experiment I, a total of 240 Hy-Line W-36 laying hens were randomly assigned to 5 treatments, eight replicates with six hens each. The treatments consisted diets containing 170g (control diet), 150 and 130g crude protein/kg, which corresponded to the essential nitrogen to non-essential nitrogen ratios of 37/63, 42/58 and 47/53, respectively. The other two treatments consisted of the same diets containing 150 and 130g crude protein/kg, but supplemented with 37.60 and 79.15g glutamic acid/kg respectively in replacement to cornstarch to equal the essential nitrogen to non-essential nitrogen ratio in control diet (37/63). Reduction on dietary crude protein by 20g/kg elicited a decrease in egg weight and egg mass, whereas the reduction on dietary crude protein by 40g/kg decreased feed intake, egg weight, egg mass and albumen weight, beyond worsening feed conversion ratio per kilogram of eggs. Layers fed diets with 150 and 130g crude protein/kg had similar performance and egg quality. Glutamic acid-added diets with 150g crude protein/kg maintained similar layer performance and egg quality to that observed in layers fed control diet. However, when added to diets with 130g crude protein/kg, glutamic acid impaired layer performance and egg quality, leading to the lowest means for variables assessed herein when compared with all treatments. Based on results, the lack of non-essential nitrogen compromises layer productivity and glutamic acid may be considered as a potential source of non-essential nitrogen when added in low concentration to low-protein diets. In experiment II, 360 Hy-Line W-36 laying hens were randomly assigned to 5 treatments, eight replicates with six hens each. Experimental diets were obtained through the graded supplementation of a mixture of L-alanine, L-glycine and glutamic acid, at the proportion of 60%,20% and 20% respectively, in a low protein diet with the essential nitrogen to non-essential nitrogen ratio of 55/45. Four more diets with the ratios of 52/48, 48/52, 44/46 and 41/59 were produced from the aforementioned low-protein diet. Additionally, a diet with a higher crude protein content was formulated to contain the essential nitrogen to non-essential nitrogen ratio of 41/59. Layers given dietary essential to non-essential nitrogen ratios of 55/45 and 52/48 had lower egg weight and worsen feed conversion ratio per kilogram of eggs compared with layers fed control diet. Egg mass was impaired only when essential to non-essential nitrogen ratios reached the 55/45. Egg quality was unaffected by dietary treatments. Based on results, the dietary essential to non-essential nitrogen ratio for laying hens must not reach 48/52.

# 1. INTRODUÇÃO GERAL

Proteínas, assim como os peptídeos e os aminoácidos provenientes de sua hidrólise desempenham papel essencial na manutenção da homeostase do organismo animal (Murray et al., 1988). Além de constituírem blocos essenciais no processo de síntese proteica, aminoácidos desempenham importantes funções secundárias no metabolismo e fisiologia do organismo animal. No passado, o atendimento das exigências de aminoácidos para galinhas poedeiras era caracterizado pelo fornecimento de rações contendo elevado teor de proteína bruta (Kumari et al., 2016). Todavia, a utilização de tal conceito na formulação de dietas para aves revela-se inviável em razão de fatores de ordem financeira, fisiológica e ambiental.

Sabe-se que os gastos com alimentação perfazem cerca de 70% do custo final de produção de aves; e que desta parcela, o maior ônus financeiro deve-se aos gastos com as fontes proteicas (Franco et al., 2016). Ao contrário de carboidratos e lipídios, a proteína fornecida em excesso não pode ser armazenada pelo organismo da ave, o que torna necessária a eliminação do nitrogênio pelo organismo na forma de ácido úrico. O processo de excreção do nitrogênio pelas aves envolve primeiramente a remoção do grupo amino dos aminoácidos em excesso. Este processo ocorre no fígado e resulta na formação do ceto-ácido referente ao aminoácido desaminado e na formação de amônia (Namroud et al., 2008), cuja conversão em ácido úrico resulta em gasto médio de energia de 6 mol de ATP/mol de aminoácido (Macleod et al., 1997).

Além do gasto energético, a excreção de nitrogênio é indesejada sob a óptica ambiental, haja visto seu potencial poluente no ar, no solo e nos recursos hídricos. Temperaturas elevadas, comumente registradas em países de clima tropical, favorecem a volatilização de uma fração do nitrogênio presente na excreta na forma de amônia, que é liberada no ar, sendo retida nas instalações e/ou liberada na atmosfera (Roberts et al., 2007). Em contato com água, a amônia pode resultar na eutrofização de recursos hídricos, levando a morte de ecossistemas aquáticos.

Frente aos malefícios acima citados, decorrentes da utilização de dietas com elevado teor de proteína bruta, é imperativo orientar a nutrição proteica para maximizar a eficiência de utilização do nitrogênio dietético de maneira a tornar mínima sua excreção, de forma a garantir adequados índices de produtividade em condições de custo mínimo. A aplicação parcial do conceito de proteína ideal em formulações de dietas para aves possibilitou a redução no aporte dietético de proteína bruta nos últimos anos. Entretanto, vale salientar que embora o fornecimento de rações com menor teor de proteína bruta, suplementadas com aminoácidos cristalinos, aumente a eficiência de utilização do nitrogênio dietético (Corzo et al., 2005), tal prática deve ser adotada com critério em função de limitações em sua aplicação como por exemplo a redução no aporte nutricional de nitrogênio, proveniente de aminoácidos não essenciais (Almeida et al., 2012).

Concomitante à redução proteica observa-se também a redução do fornecimento dietético de aminoácidos essenciais, que no caso de alguns aminoácidos essenciais, pode ser corrigido pela sua suplementação na forma cristalina. Contudo, o mesmo não ocorre com aminoácidos considerados não essenciais, que em função do percentual de redução proteica podem ser reduzidos a níveis marginais tornando-se limitantes na dieta. A deficiência destes aminoácidos pode favorecer a conversão biológica de nitrogênio essencial em nitrogênio não essencial (Novak et al., 2006), o que embora garanta a tradução da proteína, limita sua ocorrência a níveis abaixo do potencial máximo, refletindo em menor desempenho da ave.

Percebe-se que os benefícios provenientes da adoção do conceito de proteína ideal na formulação de dietas para aves, quando confrontados com os efeitos deletérios decorrentes da adoção de tal prática geram como principal resultado o seguinte questionamento: até quando é possível reduzir a proteína bruta de dietas para galinhas poedeiras sem prejudicar o desempenho da ave. Na tentativa de responder este questionamento, faz-se necessário não somente o conhecimento exato das exigências aminoacídicas das aves, como também o conhecimento da relação ideal entre nitrogênio essencial e nitrogênio não essencial que maximiza a eficiência de utilização no nitrogênio dietético. Frente ao exposto objetivou-se com a condução do presente estudo avaliar os efeitos da redução da proteína bruta em dietas suplementadas ou não com nitrogênio não essencial, assim como o efeito de diferentes relações nitrogênio essencial:nitrogênio não essencial sobre o desempenho e qualidade de ovos de galinhas poedeiras leves.

## REFERÊNCIAS BIBLIOGRÁFICAS

ALMEIDA, V.R., DIAS, A.N., BUENO, C.F.D. et al. 2012. Crude protein and metabolizable energy levels for layers reared in hot climates. **Brazilian Journal of Poultry Science** 14:203-208.

CORZO, A., FRITTS, C.A., KIDD, M.T. et al. 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. **Animal Feed Science Technology** 118, 319–327.

FRANCO, S.M., TAVERNARI, F.C., MAIA, R.C., et al. 2016. Estimation of optimal ratios of digestible phenylalanine + tyrosine, histidine, and leucine to digestible lysine for performance and breast yield in broilers. **Poultry Science** 0:1–9.

KUMARI, K.N.R., REDDY, V.R., PREETHAM, V.C., KUMAR, D.S. et al. 2016. Effect of supplementation of crystalline lysine on the performance of WL layers in tropics during summer. Trop Anim. Health Prod. 48, 705–710.

MACLEOD, M.G. Effects of amino acid balance and energy: Protein ratio on energy and nitrogen metabolism in male broiler chickens. **British Poultry Science** 38:405-411, 1997.

MURRAY, R.K.; GRANNER, D.K.; MAYES, P.A. et al. **Harper´s Biochemistry**. 21. Ed. Norwalk: Aooleton & Lange, 1988. 700p.

NAMROUD, N.F., SHIVAZAD, M., ZAGHARI, M., 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. **Poultry Science** 87:2250–2258.

NOVAK, C., YAKOUT, H. M. & SCHEIDELER, S. E. The effect of dietary protein level and total sulfur amino acid:lysine ratio on egg production parameters and egg yield in Hy- Line W-98 Hens. **Poultry Science** 85:2195-2206, 2006.

ROBERTS, S.A., XIN, H., KERR, B.J. et al. 2007. Effects of Dietary Fiber and Reduced Crude Protein on Ammonia Emission from Laying-Hen Manure. **Poultry Science** 86:1625–1632.

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# Laying hen responses to reduction on dietary crude protein supply and non-essential nitrogen supplementation

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## Abstract

We conducted this study to evaluate the effects of reduction on dietary crude protein on white commercial layer performance and egg quality. Hypothesizing that reduction on crude protein would impair layer productivity, we supplemented diets in which crude protein content was reduced with glutamic acid as a non-essential nitrogen source to determine its efficiency in warranting adequate productivity in layers fed low-protein diets. Two hundred forty 42-to-58-weeks-old Hy-Line W-36 hens were randomly assigned to five treatment groups with eight replicates and six hens per experimental unit. The treatments consisted of diets containing 170g (control diet), 150 and 130g crude protein/kg, which corresponded to the essential nitrogen to non-essential nitrogen ratios of 37/63, 42/58 and 47/53, respectively. The other two treatments consisted of the same diets containing 150 and 130g crude protein/kg, but supplemented with 37.60 and 79.15g glutamic acid/kg respectively in replacement to cornstarch to equal the essential nitrogen to non-essential nitrogen ratio in control diet (37/63). Data were analyzed as one-way ANOVA and means of treatments were compared using Tukey's multiple comparison test. Statistical significance was considered when $P \leq 0.05$. Reduction on dietary crude protein by 20g/kg elicited a decrease in egg weight and egg mass, whereas the reduction on dietary crude protein by 40g/kg decreased feed intake, egg weight, egg mass and albumen weight, beyond worsening feed conversion ratio per kilogram of eggs. Layers fed diets with 150 and 130g crude protein/kg had similar performance and egg quality. Glutamic acid-added diets with 150g crude protein/kg maintained similar layer performance and egg quality to that observed in layers fed control diet. However, when added to diets with 130g crude protein/kg, glutamic acid impaired layer performance and egg quality, leading to the lowest means for variables assessed herein when compared with all treatments. Feed conversion ratio per dozen of eggs and eggshell weight were both unaffected by dietary treatments. Based on the results, the lack of non-essential nitrogen compromises layer productivity and glutamic acid may be considered as a potential source of non-essential nitrogen when added in low concentration to low-protein diets.

*Keywords:* commercial layers, crude protein, egg quality, essential-to-total nitrogen ratio, performance.

*Abbreviations:* CP, crude protein; Glu, glutamic acid; EN, essential nitrogen; EN:NEN, essential nitrogen to non-essential nitrogen; NEAA, non-essential amino acids; NEN, non-essential nitrogen; SID, standardized ileal digestible.

## 1. Introduction

Since the major fraction of production cost is due to feed cost, the application and development of nutritional strategies, which warrant maximum economic return, are routinely one of the main concerns of poultry production industry. Protein sources are well acknowledged as the most expensive component of poultry diets. In the past, diets were formulated to meet commercial layer amino acid needs based on crude protein (CP) concept, resulting in a dietary supply of essential amino acids higher than that required by hens (Kumari et al. 2016).

Over the last decades, however, advances in molecular biology and in industrial production techniques have contributed to enhance crystalline feed-grade amino acid commercial availability at affordable prices, which consequently has led to changes in poultry diet formulation. Crystalline amino acid supplementation in commercial layer diets has shown to be an effective nutritional strategy to enhance nitrogen utilization and save feed cost without compromising poultry performance (Dozier et al. 2011).

Although the advantages aforementioned, at certain point, reduction of dietary CP content inevitably harms poultry productive performance, even meeting all essential amino acids requirements through crystalline amino acid supplementation (Namroud et al. 2008; Hernandez et al. 2012). Silva et al. (2010) reported a linear decrease in egg weight and egg mass value when dietary CP was reduced from 180 to 120g/kg diet, even providing layer requirements for all essential amino acids.

Among causes which explain the failures of low-protein amino acid supplemented-diets in reproducing similar performance to diets with higher CP content, the lack of nitrogen pool to synthesize non-essential amino acids (NEAA) remains as the most probable one (Awad et al. 2015). Studies have demonstrated that NEAA supplementation may attenuate the deleterious effects of low-CP diets on broiler performance (Corzo et al. 2005; Namroud et al. 2008; Awad et al. 2014).

Glutamic acid (Glu) is commonly supplemented in low-protein diets as a non-essential nitrogen (NEN) source, but its supplementation in broiler low-protein diets has proved to be not efficient in providing to birds similar performance to diets with a higher CP content (Corzo et al. 2005; Awad et al. 2015). In such studies, however, Glu was not supplemented to equal NEN content of low-protein to high-protein diet, but rather to maintain both diets with the same Glu content. Therefore, in a different scenario, in which Glu is added in low-protein diets in order to provide the same NEN content that high-protein diet, low-protein diets supplemented with Glu may be efficient in reproducing similar performance to diets with higher dietary CP.

Given this background, we hypothesized that the reduction on dietary CP would lead to impairments in layer performance and egg quality traits; but by adding Glu to low-CP diets such detrimental effects could be attenuated. Therefore, this experiment was conducted to evaluate the effects of CP reduction on layer performance and egg quality and the potentiality of Glu usage as a NEN source.

## 2. Material and Methods

All experimental procedures herein described were only adopted after approved by the Universidade Federal de Viçosa Institutional Animal Care and Use Committee (protocol no. 01/2015).

### *2.1. Birds, husbandry and experimental design*

Two hundred forty 42-to-58-week-old Hy-Line W-36 laying hens (body weight of 1,505 ± 45g) were used in the current study. The layers, obtained from a local commercial flock, were housed in double curtain-sided room and allotted into 68 × 48 × 38cm (length x height x depth) stainless steel cages (544cm$^2$/hen) equipped with a trough feeder and three nipple drinkers. Eight replicate cages of six hens were randomly assigned to one of five dietary treatments. Three diets differing only in their CP content were formulated to meet or exceed Rostagno et al. (2011) recommendations: 170, 150 and 130g CP/kg diet, except for CP. Diets in which CP was reduced were supplemented with crystalline amino acids to meet all requirements for essential amino acids. Since only essential amino acids were added to diets with 150 and 130 g CP/kg diet, three different EN:NEN ratios were produced from experimental diets: 37/63 (170g CP/kg diet), 42/58 (150g CP/kg diet) and 47/53 (130g CP/kg diet). Finally, the other two dietary treatments were obtained from the diets containing 150 and 130g CP/kg diet, to which Glu wa added so that both diets had equal EN:NEN (37/63) to control diet (170g CP/kg diet). Glutamic was added in replacement to cornstarch. Dietary essential nitrogen content was calculated by multiplying layer essential amino acid requirement described by Rostagno et al. (2011)

by their respective nitrogen content. Total nitrogen was estimated by multiplying nitrogen content of each feedstuff used in diet formulation by its inclusion. Finally, non-essential nitrogen was obtained by the difference between total and essential nitrogen. Crystalline amino acids were considered to be 100% SID. Throughout the 16-week feeding trial, layers were managed according to the Hy-Line W-36 Commercial Management Guide (2016) and had free access to feed (mash form) and water. Photoperiod was set at 16L:8D.

### *2.2. Performance and egg quality measurements*

Feed intake was measured every 28-day intervals throughout the experiment, whereas egg production was daily recorded. All the eggs produced at the 1st, 2nd and 3rd day of the last week of each 28-day intervals were weighed to determine egg weight. Egg mass was obtained by multiplying egg weight by egg production, and feed conversion per kilogram of eggs (kg/kg) was calculated through the division of feed intake by egg mass. Mortality was daily recorded in order to adjust feed intake, feed conversion and egg production. At 4th, 5th and 6th day of the last week of each 28-day intervals all the eggs produced were collected, identified, weighed and finally broken for egg quality trait analyses. The yolks were manually separated from the albumen and weighed to obtain the average yolk weight. The eggshells, previously identified, were dried in forced-ventilation (55 – 65C°) and weighed to determine the average eggshell weight. Albumen weight was obtained through the difference between average egg weight, yolk and eggshell weight.

### *2.3. Statistical analysis*

Data were analyzed as one-way ANOVA using PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC). When dietary treatment was significant, means were compared using Tukey’s multiple comparison test. Cages containing six hens were considered as experimental units. Statistical model included experimental treatments as fix effect and statistical significance was considered when $P \leq 0.05$.

### 3. *Results*

The effects of dietary treatments on layer performance and egg quality are detailed in Table 2 and 3, respectively. Feed intake was higher ($P<0.01$) in layers fed diets with 170g CP/kg compared with those fed 130g CP/kg diet. However, no differences were observed on feed intake of layers fed diets in which CP content was reduced (150 and 130g CP/kg diet). The supplementation of Glu in diets containing 130g CP/kg resulted in the lowest ($P<0.01$) feed intake and egg production compared with all dietary treatments. Reduction on dietary CP decreased ($P<0.01$) egg weight and egg mass regardless of the level that dietary CP was reduced. However, Glu supplementation in diets containing 150g CP/kg diet supported equal egg weight and egg mass values to diets with 170g CP/kg diet. As well as observed for feed intake and egg production, layers fed diets containing 130g CP/kg supplemented with Glu exhibited the lowest ($P<0.01$) egg mass compared with all dietary treatments. Egg weight was similar between layers fed diet 130g CP/kg regardless of the Glu supplementation. Reduction on dietary CP from 170 to 130g/kg, regardless of Glu supplementation, impaired ($P<0.01$) feed conversion ratio per kilogram of eggs. Feed conversion ratio per dozen of eggs was unaffected by dietary treatments. Albumen weight was lower ($P<0.05$) in layers fed diets containing 130g CP/kg diet supplemented or not with Glu compared with all dietary treatments, whereas yolk weight was impaired ($P<0.01$) only by Glu-supplemented diets containing 130g CP/kg. Eggshell weight was unaffected by dietary treatments.

### 4. *Discussion*

Based on previous evidences that reduction on dietary CP content, at certain levels, impairs layer productivity, and presuming that the lack of NEN is presumably the cause for such deleterious effects, we hypothesized that Glu supplementation in low-protein diets (150 and 130g CP/diet) would support equal performance and egg quality to control diet (170g CP/kg diet). Overall, reduction on dietary CP indeed impaired layer productivity, once layers fed diets with 170g/kg showed higher egg weight and egg mass versus those given diets with 150 and 130g CP/kg. Besides, the diets with 130g CP/kg decreased feed intake and worsened feed conversion ratio per kilogram of eggs.

According to Aftab et al. (2006), the impaired productivity caused by reduction on dietary CP may be due to several reasons, which include dietary potassium deficiency and altered dietary electrolyte balance caused by reduction on soybean meal; deficiency of amino acids due to incorrect supplementation of crystalline amino acids; feed intake depression; and the deficit on dietary nonessential nitrogen supply. In the current study, potassium carbonate was added to diets concurrently with the reduction on soybean meal inclusion, in order to delay dietary potassium deficiency and to maintain all diets with equal dietary electrolyte balance. Moreover, crystalline amino acids were added to diets to meet the nutritional requirements of layers for all essential amino acids. Thus, we expected to isolate the lack of NEN as the cause for depression on performance and egg quality of layers fed low-protein diets.

In the current study, reducing dietary CP in 40g (from 170 to 130g/kg) elicited a decrease by 2% in layer feed intake. CP supply has often been reported to influence both broiler (Namroud et al., 2008) and layer (Novak et al. 2006; Shim et al. 2013; Praes et al. 2014) feed intake. Although experimental diets were formulated herein to be isoenergetic on a metabolizable energy basis, it is possible that dietary CP level may have altered dietary net energy concentration, which could have influenced layer feed intake responses. Compared with carbohydrates and lipids, protein catabolism results in higher heat production on organism, reflecting in a higher heat increment (Syafwan et al. 2011).

Therefore, low-protein amino acid-supplemented diets assume a lower heat increment and consequently a higher net energy value than those from diets with a higher CP content. Once birds adjust feed intake to satisfy energy requirements (Ferket and Gernat, 2006), it is expected that the high dietary net energy concentration impacts negatively in bird feed intake behavior. Intestinal absorption rate of free amino acids, commonly added to low-protein diets, occurs faster than absorption of amino acids bound to peptides, resulting in an excessive or imbalanced amino acid profile in plasma (Aftab et al. 2006). This imbalanced amino acid profile in plasma could *per se* depress feed intake, or the ammonia derived from amino acid split in liver could serve as a trigger to activate mechanisms of appetite control (Namroud et al. 2008).

Glu is an amino acid, which has been reported as the most predominant neurotransmitter in the central nervous system involved in feed intake modulation. We noticed that Glu-supplemented diets containing 150g CP/kg did not alter layer feed intake response. However Glu-fed layers given diets with 130g CP/kg exhibited the lowest feed intake comparing all dietary treatments, which suggests that when administrated in high dietary concentration, Glu may interfere in layer feed intake. Our findings support previous studies that demonstrated an anorexic effect of Glu in some avian species (Zeni et al. 2000; Baghbanzadeh and Babapour 2007; Taati et al. 2011, Wang et al. 2012).

Presumably, the lower feed intake observed in layers fed diets containing 130g CP/kg Glu-supplemented decreased nutrient intake, which underlies the poorer performance comparing such group of layers with those fed other dietary treatments. Egg weight was impaired by CP reduction. Similarly, previous studies also demonstrated a negative correlation between egg weight and reduction of dietary CP for laying hens (Almeida et al. 2012; Moussavi et al. 2013; Ji et al. 2014). Despite egg production not be affected by diets, egg weight was impaired in layers fed 130g CP/kg diet. Such response resulted in lower egg mass values, which consequently worsened feed conversion ratio per kilogram eggs.

Regarding egg quality, our results indicated that albumen weight appears to be more sensitive than yolk weight in response to CP variation. Albumen is synthesized by oviduct for a period of approximately 3 hours, during which, the ratio of albumen protein synthesis by magnum cells is twice times higher when compared with isthmus (Hiramoto et al. 1990). Given this higher metabolic demand of magnum cells for amino acids in this short period of time, providing either insufficient or imbalanced amino acid profile may compromise albumen quality (Penz Jr and Jensen, 1991), as noticed in this current study.

Layers do not have a specific requirement for CP, which allow decreasing its content in diets supplemented with crystalline amino acids. However, dietary CP should be sufficient to ensure an adequate supply of nonessential amino acids (NRC, 1994). Overall, our results indicate that the best layer performance and egg quality was achieved in layers fed 170g CP/kg diet, which corresponded to a daily CP intake of 16.20g/hen. Similarly, Rostagno et al. (2011) recommend a daily CP intake of 16.50g/hen for optimum white commercial layers performance and egg quality, whereas Hy-Line W-36 management guide describes ideal daily CP intake for 40-to-60-week-old layers in approximately 15.50g/hen.

We noticed that Glu supplementation reproduced equal performance of layers fed diets with 150g CP/kg to layers fed 170g CP/kg diet, which corroborates the previous hypothesis herein raised that insufficient dietary NEN supply is responsible for the failure in performance and egg quality of layers fed low-protein. However, contrary to what we expected, beyond not warranting equal performance and egg quality in hens fed 130g CP/diet to layers fed control diet, Glu supplementation resulted in the worst layer performance and egg quality among treatments, presumably due to depression in layer feed intake, which suggests a possible limitation in Glu usage as NEN source.

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## REFERENCES

Aftab, U., Ashraf, M., Jiang, Z., 2006. Low protein diets for broilers. Worlds Poult. Sci. J. 62, 688-701.

Almeida, V.R., Dias, A.N., Bueno, C.F.D., Couto, F.A.P., Rodrigues, P.A., Nogueira, W.C.L., Faria Filho, D.E., 2012. Crude protein and metabolizable energy levels for layers reared in hot climates. Rev. Bras. Cienc. Avic. 14, 203-208.

Awad, E.A., Fadlullah, M., Zulkifli, I., Farjam, A.S., Chwen, L.T., 2014. Amino acids fortification of low-protein diet for broilers under tropical climate: ideal essential amino acids profile. Ital. J. Anim. Sci. 13, 270-274.

Awad, E.A., Zulkifli, I., Soleimani, A.F., Loh, T.C., 2015. Individual non-essential amino acids fortification of a low-protein diet for broilers under the hot and humid tropical climate. Poult. Sci. 94, 2772–2777.

Baghbanzadeh, A., Babapour, V., 2007. Glutamate ionotropic and metabotropic receptors affect feed intake in broiler cockerels. J. Vet. Res. 62, 125-129.

Corzo, A., Fritts, C.A., Kidd, M.T., Kerr, B.J., 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. Anim. Feed Sci. Technol. 118, 319–327.

Dozier, W.A., Corzo, A., Kidd, M.T., Tillman, P.B., Branton, S.L., 2011. Determination of the fourth and fifth limiting amino acids in broilers fed on diets containing maize, soybean meal and poultry by-product meal from 28 to 42 d of age. Br. Poult. Sci. 52, 238-244.

Ferket, P.R., Gernat, A.G., 2006. Factors That Affect Feed Intake of Meat Birds: A Review. Int. J. Poult. Sci. 5, 905-911.

Hernandez, F., Lopez, M., Martinez, S., Megias, M.D., Catala, P., Madrid, J., 2012. Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1- to 48-day-old broilers. Poult. Sci. 91, 683-692.

Hiramoto, K., Muramatsu, T., Okumura, J., 1990. Protein synthesis in tissues and in the whole body of laying hens during egg formation. Poult. Sci. 69, 264-269.

Ji, F., Fu, S.Y., Ren, B., Wu, S.G., Zhang, H.J., Yue, H.Y., Gao, J., Helmbrech, A., Qi, G.H., 2014. Evaluation of amino-acid supplemented diets varying in protein levels for laying hens. J. Appl. Poult. Res. 23, 1–9.

Kumari, K.N.R., Reddy, V.R., Preetham, V.C., Kumar, D.S., Sen, A.R., Rama Rao, S.V., 2016. Effect of supplementation of crystalline lysine on the performance of WL layers in tropics during summer. Trop Anim. Health Prod. 48, 705-710.

Moussavi, S.N., Khalaji, S., Ghasemi-Jirdehi, A., Foroudi, F., 2013. Investigation on the effects of various protein levels with constant ratio of digestible sulfur amino acids and threonine to lysine on performance, egg quality and protein retention in two strains of laying hens. Ital. J. Anim. Sci. 12, 9-15.

Namroud, N.F., Shivazad, M., Zaghari, M., 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult. Sci. 87, 2250–2258.

Novak, C., Yakout, H.M., Scheideler, S.E., 2006. The effect of dietary protein level and total sulfur amino acid:lysine ratio on egg production parameters and egg yield in HyLine W-98 Hens. Poult. Sci. 85, 2195-2206.

NRC, 1994. Nutrient Requirements of Poultry, 9th revised ed. National Academic Press, Washington, DC.

Penz Jr, A.M., Jensen, L.S., 1991. Influence of Protein Concentration, Amino Acid Supplementation, and Daily Time of Access to High- or Low-Protein Diets on Egg Weight and Components in Laying Hens. Poult. Sci. 70, 2460-2466.

Praes, M.F.F.M., Junqueira, O.M., Pereira, A.A., Filardi, R.S., Duarte, K.F., Sgavioli, S., Alva, J.C.R., Domingues, C.H.F., 2014. High-Fiber Diets with Reduced Crude Protein for Commercial Layers. Rev. Bras. Cienc. Avic. 16, 43-50.

Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C, Oliveira, R.F., Lopes, D.C.; Ferreira, A.S., Barreto, S.L.T., Euclides, R.F., 2011. Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements, third ed. Imprensa Universitária, Viçosa. http://www.lisina.com.br/arquivos/Geral%20English.pdf

Shim, M.Y., Song, E., Billard, L., Aggrey, S.E., Pesti, G.M., Sodsee, P., 2013. Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Poult. Sci. 92, 2687–2696.

Silva, M.F.R., Faria, D.E., Rizzoli, P.W., Santos, A.L., Sakamoto, M.I., Souza, H.R.B., 2010. Performance, egg quality and nitrogen balance of commercial laying hens fed diets with different levels of crude protein and lysine. R. Bras. Zootec. 39, 1280-1285.

Syafwan, S., Kwakkel, R.P., Verstegen, M.W.A., 2011. Heat stress and feeding strategies in meat-type chickens. Worlds Poult. Sci. J. 17, 653-673.

Taati, M., Nayebzadeh, H., Zendehdel, M., 2011. The effects of DL-AP5 and glutamate on ghrelin-induced feeding behavior in 3-h food-deprived broiler cockerels. J. Physiol. Biochem. 67, 217-223.

Wang, S., Khondowe, P., Chen, S., Yu, J., Shu, G., Zhu, X., Wang, L., Gao, P., Xi, Q., Zhang, Y., Jiang, Q., 2012. Effects of "Bioactive" amino acids leucine, glutamate, arginine and tryptophan on feed intake and mRNA expression of relative neuropeptides in broiler chicks. J Anim Sci Biotechnol 3, 1-8.

Zeni, L.A., Seidler, H.B., Carvalho, N.A., Freitas, C.G., Marino Neto, J., Paschoalini, M.A., 2000. Glutamatergic control of food intake in pigeons: Effects of central injections of glutamate, NMDA, and AMPA receptor agonists and antagonists. Pharmacol. Biochem. Behav. 65, 67-74.

Table 1 - Analyzed and calculated composition of experimental diets (g/kg, as-fed).

| **Ingredients, g/kg** | **Crude protein (g/kg)** | | | | |
|---|---|---|---|---|---|
| | **170** | **150** | **130** | **150 + Glu** | **130 + Glu** |
| Corn (7.82%) | 515.50 | 568.05 | 617.62 | 568.05 | 617.62 |
| Soybean meal (45.20%) | 232.87 | 202.88 | 163.17 | 202.88 | 163.17 |
| Corn gluten meal (60%) | 33.63 | 10.66 | - | 10.66 | - |
| Soybean oil | 11.16 | 6.00 | - | 6.00 | - |
| Limestone | 97.13 | 97.13 | 97.12 | 97.13 | 97.12 |
| Dicalcium phosphate | 11.66 | 11.93 | 12.27 | 11.93 | 12.27 |
| Salt | 2.61 | 2.61 | 2.60 | 2.61 | 2.60 |
| Sodium bicarbonate | 3.92 | 3.92 | 3.94 | 3.92 | 3.94 |
| Potassium carbonate | - | 0.75 | 1.81 | 0.75 | 1.81 |
| L-lysine HCl (78%) | 0.97 | 2.08 | 3.39 | 2.08 | 3.39 |
| DL-methionine (99%) | 2.47 | 3.19 | 3.75 | 3.19 | 3.75 |
| L-threonine (98%) | 0.41 | 1.19 | 1.90 | 1.19 | 1.90 |
| L-tryptophan (99%) | 0.14 | 0.36 | 0.60 | 0.36 | 0.60 |
| L-valine (96%) | 0.46 | 1.52 | 2.46 | 1.52 | 2.46 |
| L-isoleucine (98%) | - | 0.59 | 1.50 | 0.59 | 1.50 |
| L-arginine (98%) | - | - | 0.77 | - | 0.77 |
| Glutamic acid (99%) | - | - | - | 37.60 | 68.00 |
| Cornstarch | 80.00 | 80.00 | 80.00 | 42.40 | 12.00 |
| Mineral premix[1] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin premix[2] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Choline chloride | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| *Calculated composition* | | | | | |

| | | | | | |
|---|---|---|---|---|---|
| AMEn, kcal/kg | 2,800 | 2,800 | 2,800 | 2,800 | 2,800 |
| CP[3], g/kg | 170.0 | 150.0 | 130.0 | 170.14 | 170.12 |
| EN/NEN | 37/63 | 42/58 | 47/53 | 37/63 | 37/63 |
| Calcium, g/kg | 40.20 | 40.20 | 40.20 | 40.20 | 40.20 |
| Non-phytate phosphorous, g/kg | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Sodium, g/kg | 2.25 | 2.25 | 2.25 | 2.25 | 2.25 |
| Potassium, g/kg | 5.80 | 5.80 | 5.80 | 5.80 | 5.80 |
| Chloride, g/kg | 2.00 | 2.00 | 2.00 | 2.00 | 2.00 |
| DEB[4] mEq/kg | 189.79 | 189.79 | 189.79 | 189.79 | 189.79 |
| *SID amino acids, g/kg* | | | | | |
| Lysine | 8.03 | 8.03 | 8.03 | 8.03 | 8.03 |
| Methionine + cysteine | 7.31 | 7.31 | 7.31 | 7.31 | 7.31 |
| Threonine | 6.10 | 6.10 | 6.10 | 6.10 | 6.10 |
| Tryptophan | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 |
| Valine | 7.63 | 7.63 | 7.63 | 7.63 | 7.63 |
| Isoleucine | 6.51 | 6.10 | 6.10 | 6.10 | 6.10 |
| Arginine | 9.77 | 8.56 | 8.03 | 8.56 | 8.03 |
| Glycine + serine | 13.75 | 11.91 | 10.18 | 11.91 | 10.18 |
| Phenylalanine + Tyrosine | 14.01 | 11.60 | 9.67 | 11.60 | 9.67 |
| Histidine | 4.09 | 3.59 | 3.13 | 3.59 | 3.13 |
| Leucine | 15.54 | 12.69 | 10.76 | 12.69 | 10.76 |
| *Analyzed composition* | | | | | |
| CP, g/kg | 170.98 | 151.04 | 130.91 | 172.11 | 171.67 |

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[1] Provided per kilogram of diet: manganese – 65.0 mg, iron - 50.0 mg, zinc - 60.0 mg; copper - 10.0 mg, iodine - 0.80 mg, selenium - 0.30 mg and excipient.

[2] Provided per kilogram of diet: vitamin A - 10,000 IU, vitamin D3 - 2,000 IU, vitamin E - 35 IU, vitamin K3 - 1.7 mg, vitamin B6 - 2.4 mg; vitamin B12 - 12 mg, pantothenic acid - 12.0 mg, biotin - 0.07 mg, nicotinic acid - 35 g and excipient; 0.5 g choline chloride (60%) and 0.01 g butylated hydroxytoluene/kg diet.

[3] Crude protein content of diets + crude protein of glutamic acid.

[4] Dietary electrolyte balance (Mogin, 1981)

Table 2- Laying hen performance responses to dietary treatments.

| Item | | Feed intake (g/hen/day) | Egg production (g/kg) | Egg weight (g) | Egg mass (g/day) | FCR[2] (kg/kg) | FCR (kg/dozen) |
|---|---|---|---|---|---|---|---|
| Crude protein (g/kg) | EN:NEN[1] ratio (%) | | | | | | |
| 170 | 37/43 | 95.28a | 861$^{a}$ | 64.97a | 55.92a | 1.70a | 1.30 |
| 150 | 42/58 | 94.45ab | 856$^{a}$ | 62.84b | 53.82bc | 1.76ab | 1.31 |
| 130 | 47/53 | 93.37b | 839ab | 62.49bc | 52.41c | 1.78b | 1.32 |
| 150 + Glu | 37/43 | 94.25ab | 861$^{a}$ | 64.25a | 55.29ab | 1.71a | 1.30 |
| 130 + Glu | 42/58 | 90.75c | 812b | 61.42c | 49.90d | 1.82b | 1.33 |
| SEM[3] | | 0.448 | 6.77 | 0.273 | 0.513 | 0.016 | 0.011 |
| P-Value | | <0.01 | <0.01 | <0.01 | <0.01 | <0.01 | 0.209 |

Means within a column not followed by common superscript differ ($P < 0.05$).

[1] EN:TN ratio = essential nitrogen to non-essential nitrogen ratio.

[2] FCR = feed conversion ratio.

[3] SEM: standard error of the mean.

Table 3- Laying hen egg quality responses to dietary treatments.

| Item | | Albumen weight | Yolk weight | Egghell weight |
|---|---|---|---|---|
| Crude protein (g/kg) | EN:NEN[1] ratio (%) | | | |
| 170 | 37/43 | 41.14a | 17.59a | 6.18 |
| 150 | 42/58 | 39.86ab | 17.21ab | 6.10 |
| 130 | 47/53 | 38.75bc | 17.09ab | 5.97 |
| 150 + Glu | 37/43 | 40.67a | 17.54ab | 6.01 |
| 130 + Glu | 42/58 | 37.65c | 16.94b | 5.95 |
| SEM[2] | | 0.334 | 0.156 | 0.062 |
| P-Value | | <0.05 | <0.01 | 0.07 |

Means within a column not followed by common superscript differ ($P < 0.05$).

[1] EN:NEN ratio = essential nitrogen non-essential nitrogen ratio.

[2] SEM: standard error of the mean.

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# Dietary essential to non-essential nitrogen optimum ratio for white commercial layers

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## Abstract

The insufficient dietary supply of non-essential nitrogen is assumed to be responsible for the poor performance and egg quality of laying hens fed low-protein diets. In order to determine the essential to non-essential nitrogen ratio, which warrants optimum performance, and egg quality of laying hens we conducted the current study. Three hundred sixty Hy-Line W-36 laying hens were randomly assigned to one of six dietary treatments with ten replicates of six hens. A basal diet, formulated to contain a dietary essential to non-essential nitrogen ratio of 55/45, was graded supplemented with a mixture of crystalline non-essential amino acids (60% L-alanine, 20% L-glycine and 20% L-glutamic acid) to produce four more diets with the ratios of 52/48, 48/52, 44/46 and 41/59. The sixth dietary treatment, herein referred as control group, consisted of a diet formulated with a higher crude protein content with an essential to non-essential nitrogen ratio of 41/59. Data were analyzed as one-way ANOVA and means of treatments were individually compared with the control group by Dunnett's comparison test. Statistical differences were detected when $P \leq 0.05$. Layers given dietary essential to non-essential nitrogen ratios of 55/45 and 52/48 had lower egg weight and worsen feed conversion ratio per kilogram of eggs compared with layers fed control diet. Egg mass was impaired only when essential to non-essential nitrogen ratios reached the 55/45. Egg quality was unaffected by dietary treatments. Based on results, the dietary essential to non-essential nitrogen ratio for laying hens must not reach 48/52.

*Keywords:* laying hens, L-alanine, L-glutamic acid, L-glycine

*Abbreviations:* CNEAA, crystalline non-essential amino acids; CP, crude protein; Glu, glutamic acid; EN, essential nitrogen; EN:NEN, essential nitrogen to non-essential nitrogen; NEAA, non-essential amino acids; NEN, non-essential nitrogen; SID, standardized ileal digestible.

## 1. Introduction

Given the economic input, that protein sources represent on poultry feed cost; researches have been extensively focused, over the last decades; on evaluating laying hen responses to reduction on dietary crude protein (CP) content. In general, the results provide clear evidences that, at some point, the decrease in dietary CP supply inevitably harms hen performance and egg quality, even when all requirements for essential amino acid are met (Silva et al. 2010; Moussavi et al. 2013; Ji et al. 2014; Viana et al. 2014).

Based on published reports (Aftab, 2006; Namroud et al. 2008), we designed a study trying to isolate factors, which are recognized for impairing performance of hens fed decreasing CP supply, such as deficit on dietary essential amino acid supply and nutritional deficiency of potassium, in order to prove whether the low supply of non-essential nitrogen (NEN) explains poor hen performance. Indeed, we found evidences linking low NEN content in diet to impairments in performance and egg quality of laying hens fed low-protein diets (Viana et al. 2015).

The balance between dietary essential nitrogen (EN) and NEN is strongly correlated to the efficiency with which nitrogen is used by hen organism for maintenance, body protein accretion and egg protein synthesis. Thus, beyond proving that deficit on dietary NEN supply depresses poultry productivity is essential to better explain layer responses to low-protein diets, also establishing the essential nitrogen to non-essential nitrogen (EN:NEN) ratio which warrants optimum performance and egg quality of laying hens has its importance in protein and amino acid nutrition of laying hens. In previous research, we showed that increasing EN:NEN ratio from 37/63 to 42/58 decreased only white commercial layer egg weight and egg mass. However, we also noticed that layers fed diets with a EN:NEN ratio of 47/53, beyond showing lower egg weight and egg mass, also had feed intake depressed and poorer feed conversion ratio per kilogram of eggs versus those given diets containing EN:NEN ratio of 37/63 (Viana et al. 2015).

Although the study aforementioned gives an idea of the detrimental effects of decreasing NEN in diets, it is not conclusive in determining the ideal balance between EN and NEN on laying hen diets. Therefore, we conducted this study to determine the EN:NEN optimum ratio for white commercial layers in the egg-laying phase.

## 2. Material and methods

All experimental procedures herein described were only adopted after approved by the Universidade Federal de Viçosa Institutional Animal Care and Use Committee (protocol no. 01/2015).

### *2.1. Experimental diets*

A basal diet was formulated based on corn and soybean meal to contain 2,900 kcal AMEn/kg and 139.5g CP/kg. Additionally, crystalline amino acids, mineral and vitamin sources were added to basal diet in order to provide all nutritional requirements described by Rostagno et al. (2011) for white commercial layers in the egg-laying phase. Basal diet was assumed to contain a dietary EN/NEN ratio of 55/45. The estimate of dietary EN content was obtained by multiplying hen requirement for essential amino acids by their respective nitrogen content, whereas total nitrogen was estimated by multiplying nitrogen content of each feedstuff by its respective inclusion in diet. Finally, NEN was obtained through the difference between total and essential nitrogen. Other four dietary treatments were obtained from basal diet, which has its cornstarch amount graded replaced by a crystalline non-essential amino acid (CNEAA) mixture to produce dietary EN/NEN ratios of 52/48, 48/52, 44/46 and 41/59. The aforementioned CNEAA mixture added to basal diets to produce dietary treatments was compounded by 60% of L-Alanine, 20% of L-Glycine and 20% of L-Glutamic acid. Additionally, a sixth diet, with a higher CP content (180g CP/kg), was formulated to meet or exceed white commercial layer nutritional requirements (Rostagno et al., 2011) and contain equal dietary EN/NEN ratio to the diet with the higher inclusion of the CNEAA mixture (41/59). Crystalline amino acid assumed to have 100% SID.

### *2.2. Experimental design and husbandry*

Twenty-six-week-old Hy-Line W-36 laying hens were selected according to initial body weight from a local commercial flock and housed in double curtain-sided room. For two weeks, all the hens were individually housed and had their egg production rate daily measured. Finally, at 28-week-old, the hens with similar egg production were weighed and assigned to treatment groups so that body weight and egg production were similar among dietary treatments. Ten replicates with six hens each were randomly assigned to each of six dietary treatments ($n = 360$). The hens were allocated in 68 × 48 × 38 cm (length x height x depth) stainless steel cages, which provided 544 $cm^2$/hen. All the cages were equipped with two nipple drinkers and a trough feeder, which provided hens free access to water and feed (mash form) throughout the 16-week feeding trial. The minimum and maximum average daily room temperatures recorded throughout the experiment were 21 ± 3°C and 33 ± 2°C, respectively. Photoperiod was set 16L:8D.

### *2.3. Performance and egg quality measurements*

Feed intake was measured every 28-day intervals throughout the experiment, whereas egg production was daily recorded. All the eggs produced at the $1^{st}$, $2^{nd}$ and $3^{rd}$ day of the last week of each 28-day intervals were weighed to determine egg weight. Egg mass was obtained by multiplying egg weight by egg production, and feed conversion per kilogram of eggs (kg/kg) was calculated through the division of feed intake by egg mass. Mortality was daily recorded in order to adjust feed intake, feed conversion and egg production. At 4th, 5th and 6th day of the last week of each 28-day intervals all the eggs produced were collected, identified, weighed and finally broken for egg quality trait analyses. The yolks were manually separated from the albumen and weighed to obtain the average yolk weight. The eggshells, previously identified and overnight dried in forced-ventilation (55 – 65C°) were weighed to determine the average eggshell weight. Finally, albumen weight was obtained through the difference between average egg weight, yolk and eggshell weight.

### *2.4. Statistical Analysis*

All collected data were analyzed as one-way ANOVA using PROC GLM procedure of SAS (SAS Institute Inc., Cary, NC). When detected differences, the group of hens fed diet containing 180g CP/kg and EN:NEN ratio of 41/100 was individually compared with the other groups using Dunnett's comparison test. Cages containing six hens were considered as experimental units. Statistical model included experimental treatments as fix effect and statistically significance were considered for $P \leq 0.05$.

## 3. Results

The effects of EN:NEN on laying hen performance and egg quality are detailed in Table 2 and 3, respectively. Essential nitrogen to non-essential nitrogen ratios equal or higher than 52/48 decreased ($P<0.05$) egg weight and resulted in poorer ($P<0.05$) values of feed conversion ratio per kilogram of eggs. Egg mass, however, was only decreased ($P<0.05$) when the increase in EN:NEN ratio reached the value of 55/45. Feed intake, egg production and feed conversion ratio per dozen of eggs, as well as egg quality traits, were unaffected by EN:NEN ratios.

## 4. Discussion

Warranting the ideal balance between dietary essential amino acid profile and that required for maintenance and growth determines the efficiency of protein utilization and reflects on animal performance (Peres and Oliva-Teles, 2006). Based on such assumption, poultry requirements for essential amino acid have been met in more precise levels by adding crystalline essential amino acids to diets with reduced CP content. However, as well as diets must provide animals the amount of essential acids required for optimum performance, they must also supply an adequate amount of nitrogen from NEAA to ensure maximum nitrogen retention.

Since NEN sources are bound to dietary protein, it is expected that low-protein diets fortified with crystalline EAA contain lower NEN content, and therefore a higher EN:NEN ratio. In such cases, when nitrogen from NEAA like glutamic acid, glycine, proline and alanine become limiting in diets, essential amino acids may be used for non-essential purposes, which could limit protein synthesis (Novak et al. 2006; Saki et al. 2012).

In the current study, we formulated a basal diet with low CP content and high EN:NEN ratio (55/45), which were replicated and graded supplemented with a CNEAA mixture until obtaining a diet with low EN:NEN ratio (41/59), which in turn should represent a practical diet with a higher CP content. Additionally, a high-protein diet was formulated in order to reproduce the lower EN:NEN (41/59) obtained by adding crystalline non-essential amino acids mixture to the basal diet. Then, we expected that the results obtained from the current study could clarify important questions like: 1) how far dietary fraction of NEN can be decreased without compromising layer performance; and 2) whether diets formulated according to different methodologies (high-protein versus low-protein diets supplemented with NEAA) could promote equal layer performance and quality.

As we previously hypothesized, increased EN:NEN ratios impaired some layer performance characteristics. Egg production was unaffected by EN:NEN ratios, contrary to egg weight. Layers given diets in which dietary EN:NEN ratios reached values equal or higher than 52/48 had lower egg weight and worse feed conversion ratio per kilogram of eggs when a comparison is made with layers fed control diet. These results support previous findings that reduction on CP with the correspondent increase of EN:NEN are more damaging to egg weight when compared to egg production (Silva et al. 2010; Viana et al. 2014; Viana et al. 2015). In the current study, the results suggest that the dietary ratio EN:NEN ratio for layers must not exceed 48/52 to avoid performance impairments. These results are in agreement with those reported by Maia et al. (2017), who observed broiler performance impairments when EN:NEN ratios exceeded 50/50.

Contrary to we observed in a previous researches (Viana, 2014; Viana, 2015) and some published reports (Shim et al. 2013) feed intake was unaffected by decreasing in dietary CP supply and increasing in EN:NEN ratios. The intestinal absorption and metabolism of the free amino acids occurs faster when compared with amino acids bound to peptides (Aftab et al. 2006). Once in blood stream, the pool of free amino acids may cause an imbalanced plasma amino acid profile, which could depress feed intake (Austic et al. 2000). When a comparison is established between layers fed control diet with those fed equal EN:NEN ratio (41/59), we noticed no differences in performance characteristics assessed. This outcome suggests that the NEN from the CNEAA mixture and the nitrogen bound to dietary protein had equal efficiency of utilization, which may explain the reason why adding CNEAA mixture to layer diets have not interfered in layer feed intake as reported in literature. Published reports involving NEN supplementation in broiler diets also demonstrate that supplying more than one NEAA in low-protein diets, instead of adding a specific amino acid, promotes better performance beyond enhancing efficiency of nitrogen utilization (Corzo et al. 2005; Awad et al. 2014)

No effects of dietary treatments were observed on egg quality traits. Despite not reaching statistical significance, albumen weight in layers fed EN:NE ratios of 52/48 and 55/45 and were lower when compared with control group (41/59). Such numerical differences presumably may have led to the lower egg weight values observed in layers fed diets formulate to contain the aforementioned EN:NE ratios.

In the current study, we used Hy-Line W-36 hens from 28 to 44 weeks of age. According to genetic strain management guide, the recommendation of daily CP supply is around 157.50g, whereas Rostagno et al. (2011) recommends 165g. Our results demonstrated that dietary EN:NEN ratios for laying hens must not reach 48/52. On crude protein basis, the ratio aforementioned corresponded to the level of 161.15g/kg diet, which provided hens a daily CP intake of 149.92 g CP/kg diet. In conclusion,

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## REFERENCES

Aftab, U., Ashraf, M., Jiang, Z., 2006. Low protein diets for broilers. Worlds Poult. Sci. J. 62, 688-701.

Awad, E.A., Fadlullah, M., Zulkifli, I., Farjam, A.S., Chwen, L.T., 2014. Amino acids fortification of low-protein diet for broilers under tropical climate: ideal essential amino acids profile. Ital. J. Anim. Sci. 13, 270-274.

Corzo, A., Fritts, C.A., Kidd, M.T., Kerr, B.J., 2005. Response of broiler chicks to essential and non-essential amino acid supplementation of low crude protein diets. Anim. Feed Sci. Technol. 118, 319–327.

Ji, F., Fu, S.Y., Ren, B., Wu, S.G., Zhang, H.J., Yue, H.Y., Gao, J., Helmbrech, A., Qi, G.H., 2014. Evaluation of amino-acid supplemented diets varying in protein levels for laying hens. J. Appl. Poult. Res. 23, 1–9.

Maia, R.C., Rostagno, H.S., Albino, L.F., 2017. Effect of essential: total nitrogen dietary ratios using a mixture with alanine, glycine and glutamic acid on performance and blood parameters for broilers. In: International Poultry Scientific Forum.

Moussavi, S.N., Khalaji, S., Ghasemi-Jirdehi, A., Foroudi, F., 2013. Investigation on the effects of various protein levels with constant ratio of digestible sulfur amino acids and threonine to lysine on performance, egg quality and protein retention in two strains of laying hens. Ital. J. Anim. Sci. 12, 9-15.

Namroud, N.F., Shivazad, M., Zaghari, M., 2008. Effects of fortifying low crude protein diet with crystalline amino acids on performance, blood ammonia level, and excreta characteristics of broiler chicks. Poult. Sci. 87, 2250–2258.

Novak, C., Yakout, H.M., Scheideler, S.E., 2006. The effect of dietary protein level and total sulfur amino acid:lysine ratio on egg production parameters and egg yield in HyLine W-98 Hens. Poult. Sci. 85, 2195-2206.

Peres, H., Oliva-Teles, A., 2006. Effect of the dietary essential to non-essential amino acid ratio on growth, feed utilization and nitrogen metabolism of European sea bass (Dicentrarchus labrax). Aquaculture. 256, 395–402.

Rostagno, H.S., Albino, L.F.T., Donzele, J.L., Gomes, P.C, Oliveira, R.F., Lopes, D.C.; Ferreira, A.S., Barreto, S.L.T., Euclides, R.F., 2011. Brazilian tables for poultry and swine: composition of feedstuffs and nutritional requirements, third ed. Imprensa Universitária, Viçosa. http://www.lisina.com.br/arquivos/Geral%20English.pdf.

Saki, A.A., Naseri, H.R, Tabatabaei, M.M., Zamani, P., Haghight, M. Estimates of Methionine and Sulfur Amino Acid Requirements for Laying Hens using Different Models. Brazilian Journal of Poultry Science. Sept 2012/ v.14 / n.3 / 159-232

Shim, M.Y., Song, E., Billard, L., Aggrey, S.E., Pesti, G.M., Sodsee, P., 2013. Effects of balanced dietary protein levels on egg production and egg quality parameters of individual commercial layers. Poult. Sci. 92, 2687–2696.

Silva, M.F.R., Faria, D.E., Rizzoli, P.W., Santos, A.L., Sakamoto, M.I., Souza, H.R.B., 2010. Performance, egg quality and nitrogen balance of commercial laying hens fed diets with different levels of crude protein and lysine. R. Bras. Zootec. 39, 1280-1285.

Viana, G.S., Gomes, P.C., Barreto, S.L.T., Hannas, M.H., Pastore, S.M., Silva, E.A., Barbosa, L.V.S., 2014. Redução da proteína bruta em rações formuladas com conceito de proteína ideal para poedeiras leves de 24 a 40 semanas de idade. In: XII Congresso APA de Produção e Comercialização de Ovos - Associação Paulista de Avicultura.

Viana, G.S., Hannas, M.H., Rostagno, H.S., Albino, L.F.T., Barreto, S.L.T., Alves, W.J., Arnaut, P.R., 2015. Laying hens responses to non-essential nitrogen supplementation. In: XXIV Congreso de la Asociación Latinoamericana de Producción Animal (ALPA) y LX Congreso de la Sociedad Chilena de Producción Animal (SOCHIPA).

Table 1 - Calculated composition of experimental diets (g/kg, as-fed).

| **Ingredients, g/kg** | **Essential nitrogen to non-essential nitrogen ratios** | | | | | |
|---|---|---|---|---|---|---|
| | 41/59 (control) | 41/59 | 44/56 | 48/52 | 52/48 | 55/45 |
| Corn (7.82%) | 545.36 | 632.58 | 632.58 | 632.58 | 632.58 | 632.58 |
| Soybean meal (45.20%) | 246.61 | 123.33 | 123.33 | 123.33 | 123.33 | 123.33 |
| Corn gluten meal (60%) | 39.00 | 26.20 | 26.20 | 26.20 | 26.20 | 26.20 |
| Soybean oil | 37.88 | 13.48 | 13.48 | 13.48 | 13.48 | 13.48 |
| Limestone | 102.34 | 102.27 | 102.27 | 102.27 | 102.27 | 102.27 |
| Dicalcium phosphate | 12.63 | 13.85 | 13.85 | 13.85 | 13.85 | 13.85 |
| Salt | 2.08 | 0.75 | 0.75 | 0.75 | 0.75 | 0.75 |
| Sodium bicarbonate | 5.31 | 7.29 | 7.29 | 7.29 | 7.29 | 7.29 |
| Potassium carbonate | - | 3.58 | 3.58 | 3.58 | 3.58 | 3.58 |
| L-lysine HCl (78%) | 1.18 | 5.13 | 5.13 | 5.13 | 5.13 | 5.13 |
| DL-methionine (99%) | 2.67 | 4.13 | 4.13 | 4.13 | 4.13 | 4.13 |
| L-threonine (98%) | 0.50 | 2.50 | 2.50 | 2.50 | 2.50 | 2.50 |
| L-tryptophan (99%) | 0.18 | 0.90 | 0.90 | 0.90 | 0.90 | 0.90 |
| L-valine (96%) | 0.55 | 3.11 | 3.11 | 3.11 | 3.11 | 3.11 |
| L-isoleucine (98%) | - | 2.08 | 2.08 | 2.08 | 2.08 | 2.08 |
| L-arginine (98%) | - | 2.13 | 2.13 | 2.13 | 2.13 | 2.13 |
| CNEAA mixture[1] (99%) | - | 51.75 | 35.97 | 22.43 | 10.79 | - |
| Cornstarch | - | 1.25 | 17.03 | 30.57 | 42.21 | 5.30 |
| Mineral premix[2] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Vitamin premix[3] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Choline chloride | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 | 0.40 |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
| *Calculated composition* |  |  |  |  |  |  |
| AMEn, kcal/kg | 2,900 | 2,900 | 2,900 | 2,900 | 2,900 | 2,900 |
| CP[4], g/kg | 181.00 | 189.47 | 174.23 | 161.15 | 149.92 | 139.50 |
| EN/TN ratio | 41/59 | 41/59 | 44/56 | 48/52 | 52/48 | 55/45 |
| Calcium, g/kg | 42.30 | 42.30 | 42.30 | 42.30 | 42.30 | 42.30 |
| Non-phytate phosphorous, g/kg | 3.23 | 3.23 | 3.23 | 3.23 | 3.23 | 3.23 |
| Sodium, g/kg | 2.42 | 2.42 | 2.42 | 2.42 | 2.42 | 2.42 |
| Potassium, g/kg | 6.24 | 6.24 | 6.24 | 6.24 | 6.24 | 6.24 |
| Chloride, g/kg | 2.15 | 2.15 | 2.15 | 2.15 | 2.15 | 2.15 |
| DEB[5] mEq/kg | 204.20 | 204.20 | 204.20 | 204.20 | 204.20 | 204.20 |
| *SID amino acids, g/kg* |  |  |  |  |  |  |
| Lysine | 8.66 | 8.66 | 8.66 | 8.66 | 8.66 | 8.66 |
| Methionine + cysteine | 7.88 | 7.88 | 7.88 | 7.88 | 7.88 | 7.88 |
| Threonine | 6.58 | 6.58 | 6.58 | 6.58 | 6.58 | 6.58 |
| Tryptophan | 1.99 | 1.99 | 1.99 | 1.99 | 1.99 | 1.99 |
| Valine | 8.23 | 8.23 | 8.23 | 8.23 | 8.23 | 8.23 |
| Isoleucine | 6.98 | 6.58 | 6.58 | 6.58 | 6.58 | 6.58 |
| Arginine | 10.41 | 8.66 | 8.66 | 8.66 | 8.66 | 8.66 |
| Glycine + serine | 14.70 | 19.80 | 17.00 | 14.29 | 11.96 | 9.80 |
| Phenylalanine + Tyrosine | 15.07 | 10.22 | 10.22 | 10.22 | 10.22 | 10.22 |
| Histidine | 4.37 | 3.02 | 3.02 | 3.02 | 3.02 | 3.02 |
| Leucine | 16.81 | 12.36 | 12.36 | 12.36 | 12.36 | 12.36 |
| *Analyzed composition* |  |  |  |  |  |  |
| CP, g/kg | 185.90 | 191.80 | 179.95 | 167.75 | 151.45 | 140.10 |

[1] Crystalline non-essential amino acids mixture (60% alanine, 20% glycine and 20% glutamic acid): 965.6g CP/kg. Crystalline amino acids were assumed to have 100% SID.

[2] Provided per kilogram of diet: manganese – 65.0 mg, iron - 50.0 mg, zinc - 60.0 mg; copper - 10.0 mg, iodine - 0.80 mg, selenium - 0.30 mg and excipient.

[3] Provided per kilogram of diet: vitamin A - 10,000 IU, vitamin D3 - 2,000 IU, vitamin E - 35 IU, vitamin K3 - 1.7 mg, vitamin B6 - 2.4 mg; vitamin B12 - 12 mg, pantothenic acid - 12.0 mg, biotin - 0.07 mg, nicotinic acid - 35 g and excipient; 0.5 g choline chloride (60%) and 0.01 g butylated hydroxytoluene/kg diet.

[4] Crude protein content of diets + crude protein of crystalline amino acids.

[5] Dietary electrolyte balance (Mogin, 1981).

Table 2 – Performance of laying hens fed different essential nitrogen to non-essential nitrogen ratios.

| EN:NEN[1] ratio | Feed intake (g/hen/day) | Egg production (g/kg) | Egg weight (g) | Egg mass (g/day) | FCR[2] (kg/kg) | FCR (kg/dozen) |
|---|---|---|---|---|---|---|
| 41/59 (control) | 92.66 | 935 | 59.44a | 55.95a | 1.67a | 1.12 |
| 41/59 | 92.30 | 931 | 58.03a | 54.03a | 1.71a | 1.15 |
| 44/56 | 93.49 | 937 | 57.96a | 54.33a | 1.72a | 1.15 |
| 48/52 | 93.79 | 938 | 58.08a | 54.47a | 1.72a | 1.15 |
| 52/48 | 93.70 | 939 | 57.27b | 53.77a | 1.74b | 1.15 |
| 55/45 | 92.47 | 914 | 56.90b | 51.97b | 1.78b | 1.13 |
| SEM[3] | 0.696 | 8.14 | 0.512 | 0.657 | 0.020 | 0.015 |
| P-value | | | | | | |
| Diet effect | 0.99 | 0.24 | <0.01 | <0.01 | <0.01 | 0.99 |

Means within a column not followed by common superscript differ (*P* < 0.05).

[1] EN:NEN ratio = essential nitrogen to non-essential nitrogen ratio.

[2] FCR = feed conversion ratio.

[3] SEM: standard error of the mean.

Table 3 – Egg quality of laying hens fed different essential nitrogen to non-essential nitrogen ratios.

| EN:NEN[1] ratio | Albumen weight (g) | Yolk weight (g) | Eggshell weight (g) |
|---|---|---|---|
| 41/59 (control) | 38.71 | 15.70 | 5.38 |
| 41/59 | 37.29 | 15.65 | 5.40 |
| 44/56 | 37.37 | 15.88 | 5.36 |
| 48/52 | 37.41 | 16.00 | 5.40 |
| 52/48 | 37.25 | 15.90 | 5.39 |
| 55/45 | 36.60 | 15.71 | 5.35 |
| SEM[2] | 0.481 | 0.158 | 0.062 |
| P-value | | | |
| Diet effect | 0.06 | 0.99 | 0.99 |

[1] EN:TN ratio = essential nitrogen to non-essential nitrogen ratio.

[2] SEM: standard error of the mean.

# ANEXO I

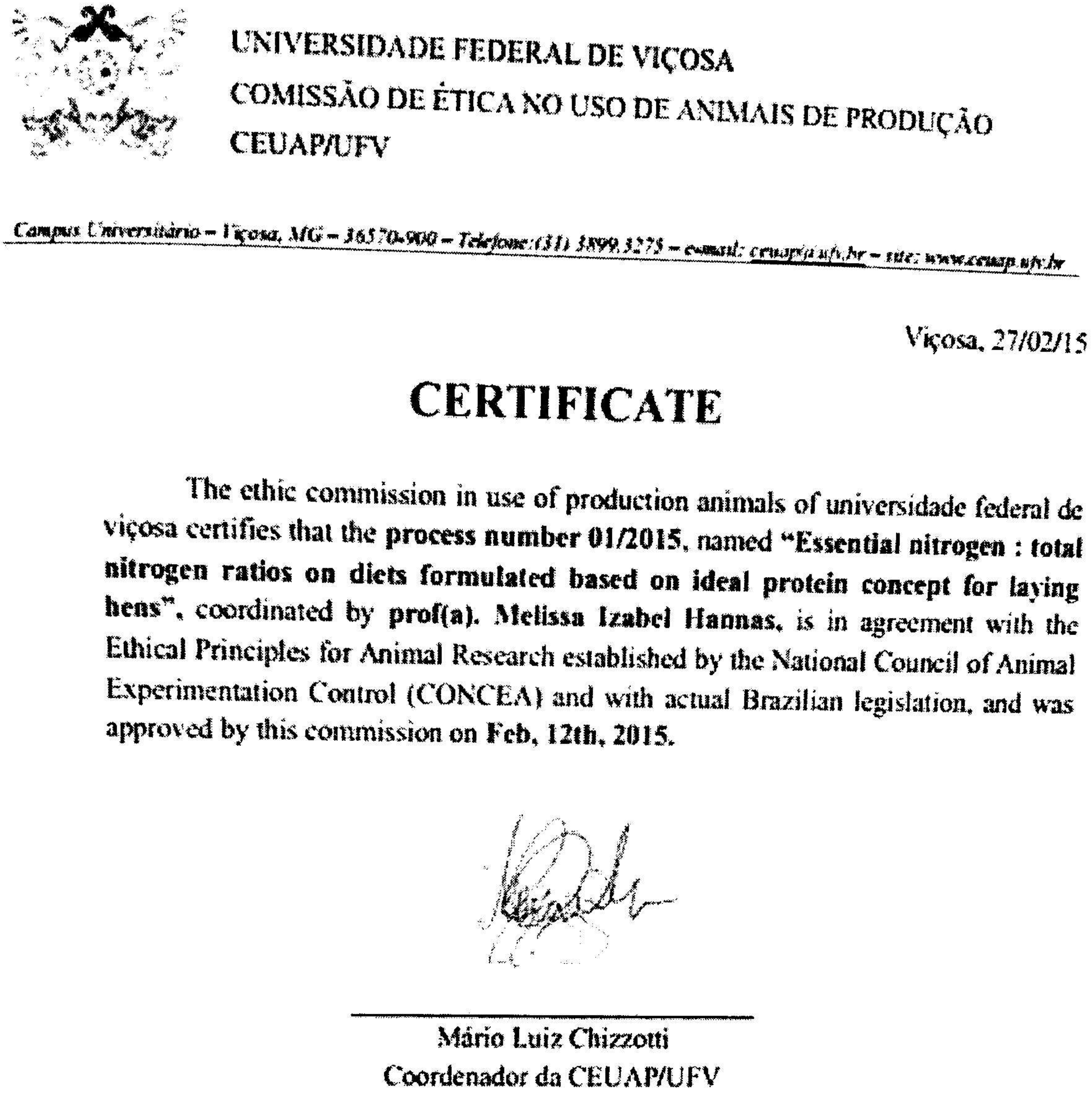
UNIVERSIDADE FEDERAL DE VIÇOSA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS DE PRODUÇÃO
CEUAP/UFV

*Campus Universitário – Viçosa, MG – 36570-900 – Telefone:(31) 3899.3275 – e-mail: ceuap@ufv.br – site: www.ceuap.ufv.br*

Viçosa, 27/02/15

# CERTIFICADO

A comissão de ética no uso de animais de produção da universidade federal de viçosa certifica que o **processo nº 01/2015**, intitulado **"Relações nitrogênio essencial : nitrogênio total em dietas formuladas com conceito de proteína ideal para galinhas poedeiras leves"**, coordenado pelo **prof(a). Melissa Izabel Hannas**, está de acordo com os princípios éticos da experimentação animal, estabelecido pelo Conselho Nacional de Controle de Experimentação Animal - CONCEA e com a legislação vigente, tendo sido aprovado por esta Comissão em **12/Fev/2015**.



UNIVERSIDADE FEDERAL DE VIÇOSA
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Viçosa, 27/02/15

# CERTIFICATE

The ethic commission in use of production animals of universidade federal de viçosa certifies that the **process number 01/2015**, named **"Essential nitrogen : total nitrogen ratios on diets formulated based on ideal protein concept for laying hens"**, coordinated by **prof(a). Melissa Izabel Hannas**, is in agreement with the Ethical Principles for Animal Research established by the National Council of Animal Experimentation Control (CONCEA) and with actual Brazilian legislation, and was approved by this commission on **Feb, 12th, 2015.**

Mário Luiz Chizzotti
Coordenador da CEUAP/UFV