

UNIVERSIDADE FEDERAL DE VIÇOSA

ROSANA CARDOSO MAIA

**RELAÇÕES DE NITROGÊNIO ESSENCIAL E TOTAL EM DIETAS DE BAIXA  
PROTEÍNA PARA FRANGOS DE CORTE**

VIÇOSA – MINAS GERAIS

2017

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

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**Luiz Fernando Teixeira Albino**  
(Orientador)  
*In memoriam*

## **DEDICO**

... À Deus que sempre se faz presente em minha vida,  
À minha família pelo apoio em todas as decisões,  
Aos meus amigos aos quais compartilhei grandes momentos durante todo o doutorado,  
Aos mestres e professores por me proporcionarem oportunidades de me desenvolver e  
aprender...

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## **EPIGRAFE**

“Dificuldades e obstáculos são fontes valiosas de saúde e força para qualquer sociedade.”

*Albert Einstein*

## **BIOGRAFIA**

Rosana Cardoso Maia, nasceu em Belo Horizonte em 1987, filha de Marcos Antonio Maia e Maria Fontana Cardoso Maia

Em Maio de 2006 iniciou o curso de Zootecnia na Universidade Federal de Viçosa, concluindo-o em Janeiro de 2011.

Em Fevereiro de 2011 iniciou o programa de Pós Graduação na Universidade Federal de Viçosa, em nível de Mestrado, como sub área nutrição aminoacídica para frangos de corte com ênfase em aminoácidos de cadeia ramificada, sendo concluído em Janeiro de 2013.

Em Fevereiro de 2013 iniciou o Doutorado na mesma instituição, como foco principal em relações de nitrogênio essencial e não essencial para frangos de corte. Em 2015 foi aprovada pelo programa de doutorado sanduíche no exterior onde esteve por 1 ano na Universidade de Purdue / IN sob a supervisão do professor Todd Applegate.

Em 2016 retomou as pesquisas de doutorado na UFV e auxiliou na elaboração das Tabelas Brasileiras de Composição de Alimentos e Exigências Nutricionais para Aves e Suínos, lançadas em março de 2017.

Assumiu o cargo de Nutricionista Animal na Empresa BRF em Abril de 2017, responsável pela nutrição de aves e suínos no estado do Rio Grande do Sul.

## RESUMO

MAIA, Rosana Cardoso, M.Sc., Universidade Federal de Viçosa, dezembro de 2017.

**Relações de nitrogênio essencial e total em dietas de baixa proteína para frangos de corte.**

Orientador: Luiz Fernando Teixeira Albino (*In memoriam*). Coorientadores: Horacio Santiago Rostagno e Arele Arlindo Calderano

Foram realizados quatro experimentos no Setor de Avicultura do Departamento de Zootecnia da Universidade Federal de Viçosa com o objetivo de avaliar diferentes relações de nitrogênio essencial:total (Ne:Nt) para frangos de corte de 8 a 21 dias e seus efeitos no desempenho, nos parâmetros sanguíneos e na utilização do nitrogênio. No experimento 1 foram utilizados 150 frangos de corte machos Cobb<sup>®</sup> 500 com peso inicial de 193g distribuídos em três tratamentos. O tratamento 1 consistiu de um controle positivo com 22,25% PB e 47,66% Ne:Nt; tratamento 2 de um controle negativo com 20,5%PB e 51,48% Ne:Nt; e tratamento 3 consistiu do T2 suplementado com ácido glutâmico (Glu) até atingir a mesma relação do controle positivo (47,66%). No experimento 2 foram avaliadas outras fontes de aminoácidos não essenciais como doadoras de nitrogênio não específico em dietas com baixo teor proteico para frangos de corte. Aos 8 dias de idade, 300 aves com peso inicial 206g foram distribuídas a 6 tratamentos compostos por: T1, controle positivo 22,25%PB, T2, uma dieta basal controle negativo com 18,5%PB, os demais tratamentos foram formados pela substituição de amido pelos aminoácidos para atender as relações semelhantes ao controle positivo: T3, T2 + Glicina (Gly), T4, T2+ Glu, T5, T2+Alanina (Ala) e T6, T2+Gly+Glu+Ala. Com os resultados do experimento 2, o experimento 3 foi conduzido. Aos 8 dias de idade 300 aves com peso médio inicial de 185g foram distribuídas em 6 tratamentos para avaliar por dose resposta a melhor relação Ne:Nt em rações de baixa proteína. Foi utilizado um tratamento controle positivo semelhante à uma dieta comercial com 47% Ne:Nt, e 5 tratamentos subsequentes com diferentes relações Ne:Nt (56%, 53%, 50%, 47% e 44%) suplementados com uma mistura de aminoácidos não essenciais compostos por 60% Ala, 20% Glu e 20% Gly. No Exp.4 foi avaliado a suplementação de nitrogênio proteico e não proteico em rações para frangos de corte em arranjo fatorial 2x2 em uma dieta basal com 19%PB ou 56%Ne:Nt. A adição de Glu, ureia ou a combinação de Glu e ureia foi feita de modo que os tratamentos atingissem uma relação de 50% de Ne:Nt. Em todos os experimentos foram utilizados 10 repetições com 5 aves, ração e água foram fornecidas *ad libitum* e as dietas foram formuladas à base de milho, gluten de milho e farelo de soja para atender a recomendação nutricional de Rostagno *et al.*, (2011) com exceção da proteína. Para a análise estatística dos dados foi utilizado o pacote estatístico SAS 9.2 PROC GLM, PROC REG

e PROC NLIN. A comparação de tratamentos controles com os demais foi realizada pelo teste de Dunnett ( $P < 0,05$ ). No experimento 1 o uso de Glu diminuiu o consumo de ração e melhorou a conversão alimentar em dietas com 20,5%PB. A concentração de ácido úrico no soro foi superior com a suplementação de Glu. No experimento 2 o uso de Glu, Ala ou a mistura Gly+Glu+Ala melhorou a conversão alimentar de frangos alimentados com dietas de 19% PB, entretanto a suplementação individual de glicina apresentou efeito tóxico ao nível de 3,97% de L-Gly suplementada. No experimento 3 uma resposta não linear foi encontrada para as diferentes relações de Ne:Nt, utilizando o modelo LRP, a melhor relação estabelecida tanto para desempenho quanto parâmetros sanguíneos foi próxima à 50%. No experimento 4 a suplementação de ureia em rações com baixa proteína proporcionou um efeito negativo no desempenho das aves, ao passo que a o Glu ou a combinação de Glu e ureia melhorou a conversão alimentar quando comparada à dieta 19%PB. Em conclusão, a redução proteica das dietas deve ser feita de forma criteriosa respeitando o aporte de nitrogênio não essencial. É recomendado que as dietas não ultrapassem valores de 50% Ne:Nt. As aves possuem baixa capacidade de utilização de nitrogênio não proteico, e o uso de nitrogênio proteico representa uma alternativa como fonte de nitrogênio não específico em rações de frangos de corte.

*Abreviações: Ne – Nitrogênio essencial; Nt – Nitrogênio Total, Glu – Ácido Glutâmico, Gly – Glicina ; Ala – Alanina.*

## ABSTRACT

MAIA, Rosana Cardoso, M.Sc., Universidade Federal de Viçosa, December 2017. **Essential-to-total nitrogen ratio in low-protein diets for broilers chickens.** Advisor: Luiz Fernando Teixeira Albino (*In memoriam*). Co-Advisors: Horacio Santiago Rostagno and Arele Arlindo Calderano

There were conducted four experiment in the Poultry Farm from Animal Science Department at Universidade Federal de Viçosa to evaluate different essential-to-total nitrogen ratio (eN-to-tN) for broilers chickens and their effects on performance, blood parameters and nitrogen utilization. In Exp.1, 150 broilers were used and allocated to three treatments. Treatment 1 was a positive control of 22.25%CP and 47.66% of eN-totN. Treatment 2 was a negative control with 20.5% CP and 51.48% eN-to-tN and treatment 3 was T2 added Glu until meet the same nitrogen ratio from positive control. During Exp.2, non-essential amino acids sources were evaluate, 300 broiler were used in and allocated to 6 treatments. T1 was a positive control with 22.25% CP, T2, a basal diet negative control with 18.5%CP, and other treatments were compounds by the replacement of amino acids to corn starch on basal diet to meet similar nitrogen ratio from positive control. T3, T2 + Gly, T4, T2 + Glu, T5, T2 + Ala, T6, T2 + Gly+Glu+Ala. With the results obtained in Exp.2, an Exp 3. were designed. 300 broilers were used in 6 treatments to measure trough response dose the best eN-to-tN ratio in low CP diets. It was used a positive control similar a commercial diet with 47% of eN-to-tN, and subsequently five treatments with different eN-to-tN ratio (56%, 53%, 50%, 47% and 44%) supplemented with a mixture of non-essential amino acids (60%Ala, 20%Glu, 20%Gly). In the Exp.4 it was evaluated the protein and non-protein sources for broilers fed low CP diets in factorial arrangement 2x2 using a basal diet with 19% of CP or 56% of eN-to-tN ratio. The addition of Glu, urea or the combination of Glu and Urea were done unti the treatments meet a 50% eN-to-tN ratio. For all experiments was used 10 replicates with five birds, food and water were given ad libitum and diets were formulated with corn, corn gluten meal, and soybean meal 46% to meet the nutritional requirements, except for protein (Rosatagno *et al.*, 2011). For statistical analysis it was used the SAS 9.2 PROC GLM, PROC REG and PROC NLIN. To compare control treatments with others, it was used a Dunnett test (P<0.05). The experimental results showed a big efficacy of the use of non-essential amino acids as a donor of non-specific nitrogen. Exp 1. The Glu decrease FI and improve FCR in chicks fed a 20.5%CP diets. The serum uric acid was higher with Glu supplementation. Exp 2. The use of Glu, Ala or the mixture improve FCR from broilers fed 19%CP leves. However the individually use of Gly showed a toxic effect at level of 3.96% of L-Gly supplementation. Exp. 3 A non-linear response was

found for eN-to-tN ratio, using a LRP model, the best ratio established for performance and blood parameters were close to 50%. Exp.4 the urea supplementation in low CP diets showed a negative effect on performance , whereas the Glu supplementation or Glu+ Urea improved FCR when compared to low CP diet. In conclusion, the protein reduction in diets should be done in a criteria way, respecting the apport of non-essential nitrogen. It is recommended eN-to-tN ratio similar or lower than 50% to maintain broilers performance as birds fed high CP levels. Birds have lower capacity of non-protein nitrogen utilization, however, essential amino acids showed a good alternative as non-specific nitrogen source for broilers diet.

*Abbreviations: eN – Essential Nitrogen; tN – Total Nitrogen; Glu – Glutamic Acid; Gly – Glycine; Ala – Alanine.*

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## INTRODUÇÃO GERAL

Os aminoácidos são peças fundamentais na formação da proteína. Na literatura eles são classificados como essenciais (AAE), àquele ao qual o animal não produz ou produz em taxas insuficientes para manutenção e crescimento, e os não essenciais (AANE) àqueles que o animal é capaz de produzir e sem necessidade de grandes cuidados no aporte dietético. Porém, o conceito de essencialidade tem-se tornado cada vez mais dinâmico.

Mitchell e Scott, 1951 conceitualizaram a proteína ideal em rações para frangos de corte, caracterizada pelo balanço exato de AAE sem sobra ou deficiência para manutenção e ganho. Porém nenhuma relação foi feita para os AANE devido à falta de informações. Durante os anos de 60 e 70, várias pesquisas realizadas resultaram em modificações dos padrões de requerimento de aminoácidos para frangos, com a adoção de um conceito de essencialidade condicional. A partir de então intensificaram o uso de AANE em dietas com resultados eficazes em desempenho. Entretanto o uso de alguns AANE, como o Ácido Glutâmico, tinha como objetivo manter dietas experimentais isonitrogênicas e pouco se pesquisava a respeito dos requerimento de AANE. Wu *et al.*, 2014 publicaram o primeiro estudo sobre a relação de AANE na proteína ideal. Os autores encontraram os respectivos valores em % da lisina: Alanina (102%), asparagina (56%), ácido aspártico (66%), glutamina (128%), ácido glutâmico (176%) e prolina (60%).

A utilização de dietas com baixo teor proteico e suplementação com AAE nem sempre são eficientes em alcançar o mesmo desempenho proporcionado por dietas de alta proteína. Um ponto importante a ser destacado é que existe a possibilidade de que à medida que se reduz o teor de proteína, os AANE se tornam um fator limitante nas rações. Com isso a definição de uma ótima relação AAE : AANE em dietas com baixo teor de proteína poderia ser importante para obter um ótimo desempenho e eficiência na utilização da proteína (D'Mello, 2003).

A relação entre aminoácidos pode ser calculada de diversas maneiras, como relações de AAE com aminoácido total (Ikemoto *et al.*, 1989), AANE (Mitchell *et al.*, 1968) ou proteína total (Bedford and Summers, 1985). Entretanto, existe um questionamento em calcular relações em bases aminoacídicas, isso porque, os AANE possuem uma variação no conteúdo de nitrogênio, por exemplo, Glu (9,52%), Gln (19,17%), Asp (10,52%), Asn (21,20%), Ala (15,72%) e Gly (18,66%) (Rostagno *et al.*, 2017). Ou seja, existe entre eles uma variação de até 56% no aporte de nitrogênio que deve ser levado em consideração nas formulações de rações.

A melhor forma de expressar relações de nitrogênio é através da proporção nitrogênio essencial (NE): nitrogênio total (NT), apesar do conceito ainda apresentar certa divergência na quantificação de nitrogênio e aminoácidos essenciais. Alguns trabalhos consideram como AAE o somatório do grupo na composição dietética (Ospina-Rojas *et al.*, 2012), enquanto que outros consideram como essenciais os valores da exigência do animal (Maia *et al.*, 2016). Segundo Maia *et. al.*, 2017 a essencialidade de um aminoácido é uma característica inerente ao animal ao passo que a totalidade de aminoácido é inerente à dieta. O AAE que se encontra em excesso na formulação é considerado essencial até atingir a quantidade do requerimento, acima dele é considerado AANE. Os cálculos de NE são realizados através da ponderação da exigência do AAE e a concentração de nitrogênio na molécula.

A variação na determinação de NT pode estar relacionada ao modelo de quantificação de proteína. A metodologia de determinação da proteína é realizada pela obtenção do teor de nitrogênio através dos métodos de Kjeldahl ou Dumas. O conteúdo de nitrogênio é multiplicado pela constante 6,25% seguindo duas premissas: a) todo o nitrogênio quantificado provém da proteína; b) toda proteína contém 16% de nitrogênio. Porém sabemos que parte do nitrogênio não provém de moléculas proteicas, como ácidos nucleicos, aminas e aminoácidos não proteicos (Gomes & Oliveira, 2011) e que nem toda proteína possui 16% de nitrogênio, isso porque o teor de nitrogênio depende do perfil de aminoácidos que compõe a proteína. Diante

da segunda premissa, alguns autores recomendam o uso de um fator de conversão específico para cada ingrediente (Sriperm, Pesti, Tillman, 2010), porém a falta de informações na literatura limita a aplicação desse novo conceito. Atualmente segue-se a correção recomendada pela FAO, 2003.

Considerando esses novos conceitos de relações de nitrogênio, objetivou-se com os trabalhos a seguir avaliar o uso de fontes proteicas e não proteicas em dietas com redução proteica e determinar a qual relação NE:NT podemos reduzir a proteína para manter o desempenho semelhante à uma dieta com alto teor proteico.

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SOURCES OF NON ESSENTIAL AMINO ACIDS FOR BROILERS FED LOW PROTEIN DIETS

**Effect of dietary non-essential amino acids sources on performance, nitrogen utilization and blood parameters for broilers**

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**ABSTRACT** Two trials were conducted to evaluate the effect of supplementation of non-essential amino acids (NEAAs) on performance and blood parameters of Cobb-500<sup>®</sup> broilers from 8 to 21 d of age. In experiment 1, 150 broilers chicks were divided to three treatments where glutamic acid (Glu) was the only NEAAs source. The treatments consisted of two eN:tN ratio (47.66 and 51.48%) and Glu supplementation in low protein diet to reach the same essential-to-total nitrogen(eN:tN) ratio as a control diet. In trial 2, 300 birds from 8 to 21d old were allocated into six treatments consisted of eN:tN ratio: T1 = positive control diet 22.25 % CP (47.06%); T2 = negative control diet with 18.5% CP (56.95% eN:tN); T3 = T2+ Glycine (47.06% of eN:tN); T4 = T2+ Alanine (47.06% of eN:tN); T5 = T2 + Glu (47.06% of eN:tN); T6 = T2+ Mixture (Gly+Ala+Glu) (47.06% of eN:tN). Growth performance and blood parameters data were analyzed using proc GLM (SAS 9.4), and compared by dunnett test and SNK test with alpha of 0.05%. Weight gain and feed conversion ratio worsened with the reduction of CP in diet; the NEAAs supplementation was able to recover performance with exception to the birds that received Gly. The nitrogen retention was higher for positive control than negative control diets, while the serum uric acid was higher for treatments supplemented with NEAA. As the protein was reduced, the eN:tN ratio was increased, that leads to a limit in decrease dietary CP until NEAAs become limiting. When Glu, Ala or the Mix (Gly+Ala+Glu) are used in low CP diets, they can increase broilers performance.

*Keywords:* nitrogen, essential amino acids, poultry.

*Abbreviations:* AA, amino acids; EAA, essential amino acids; NEAA, non-essential amino acids;

*N, nitrogen; eN, essential nitrogen; tN, total nitrogen; NEN, non-essential nitrogen*

## INTRODUCTION

Essential amino acids are described as amino acids which animals cannot produce or they cannot synthesize at its maximum performance, therefore these amino acids should be added to the diet, while non-essential amino acids can be produced by animals and don't need to be added to the diet. Recent researches have shown that the amino acid essentiality concept is dynamic in all animal species, and it should be influenced by different factors as protein levels in diet, age, sanitary status, sex, specie and others (Wu, 2014).

The protein levels in a diet is important to evaluate the essentiality of the called "non-essential amino acid", because when we reduce the protein level, we also reduce the non-specific nitrogen that could be used for the synthesis of non-essential amino acid. This means that a big reduction in protein level will limit non-essential amino acid synthesis. Since the synthesis should be constant in the body, part of the essential amino acid can be diverted from protein synthesis to catabolism playing a role as nitrogen source. There is a lot of research about the proportion of EAA but a lack of information about NEAA even though more than a half of protein supply is from NEAA.

Glycine, alanine, aspartate, asparagine, glutamate, glutamine and proline are known as non-essential amino acids. Some researches have shown that broilers chicks have higher concentration of glycine and glutamate in muscles, leading to higher quantity of these to synthesize protein (Wu, 2014). Ospina-Rojas et al., 2013 have shown that glycine and glutamate deficiency is most likely the cause of decreased performance in animals fed with low protein diets.

In some researches the addition of glycine, alanine or glutamate could fully recover weight, feed conversion, or both, in broilers fed low-protein diets (Ospina-Rojas, 2013, Hager and Pack, 1996, Dean et al., 2006; Maia, R.C et al., 2016). Therefore the ideal protein concept has been changed for amino acid ratios. Stranks et al., (1988) defined the ideal protein as the one having a

“correct balance between the essential and non-essential amino acids” because the intact protein in diet should supply non-essential amino acids until a limit, above this limit a source of non-essential amino acid should be added in the diets.

In this context, two experiments were carried out to evaluate sources of non essential amino acids in low protein diets and their effects on performance, blood parameters and nitrogen retention in broilers chicks.

## **MATERIALS AND METHODS**

The experiment procedures were previously approved by the Ethics Committee on Animal Use and conducted according to the experimental protocol for use of live birds (Brazilian College of Animal Experimentatio, potocol N°. 106/2014).

Two experiments were carried out in the Poultry Farm at Federal University of Viçosa with a total of 510 male Cobb-500 broiler chicks used during the starter phase (8 to 21 d of age). The chickens were raised until 8d of age on a masonry house divided into small brooding areas with wood-shavings litter, tube feeders and manual drinkers with ad libitum access to food and water. The lighting program consisted of 24L and temperature was kept at 32°C in the first week and gradually reduced according the Cobb® manual recommendations. A corn and soybean meal pre-starter diet was formulated to meet the nutrients recommendation proposed by Rostagno et al., 2011. On d 8 the birds were individually weighed and randomly allotted to dietary treatments and placed into battery cages (0.32m<sup>2</sup>/ bird), each cage with 5 birds.

In the first experiment, three dietary treatments were fed to 10 replicates of seven birds each. The first treatment was a positive control diet with 22.25% of crude protein (CP) and 47.66% of essential-to-total nitrogen ratio (eN to tN), the second treatment was a negative control diet with 20.5% of CP and 51.48% of eN-to-tN, the third treatment was the negative control

supplemented with glutamic acid to meet the same eN-to-tN ratio of positive control of 47.66% (Table 1). In the second experiment, six dietary treatments were fed to 10 replicates of five birds each. Treatments consisted of six eN-to-tN ratio: T1 = positive control diet 22.25 % of CP (47.06%); T2 = negative control diet with 18.5% of CP (56.95% of eN-to-tN); T3 = T2+ Glycine (47.06% of eN-to-tN); T4 = T2+ Alanine (47.06% of eN-to-tN); T5 = T2 + Glutamic Acid (47.06% of eN-to-tN); T6 = T2+ Mixture (Gly+Ala+Glu) (47.06% of eN-to-tN) (Table 2). Treatments were obtained by supplementing the basal diet with non essential amino acid as replacement for the corn starch. All diets were supplemented with 1% of insoluble acid ash (IAA) to measure the nitrogen balance. The nitrogen ratio was calculated in all diets considering the requirement of essential amino acids multiplied by their nitrogen content. The total nitrogen was calculated through the sum of nitrogen content in the diet feedstuffs composition.

Researches in the literature suggested that the reduced dietary electrolyte balance (DEB) of low CP diets might contribute to a portion of the reduction in growth performance (Fancher and Jensen, 1989a). To avoid the effects of different DEB, we used potassium carbonate and sodium bicarbonate to keep the DEB constant at 200 mEq/kg of diet.

At 14 d of age, the collection of excreta was performed twice daily to avoid fermentation and nutrient losses. Excreta samples were collected in plastic bags and placed in a freezer 20°C. After the collection period, they were defrosted, homogenized and dried in a 55°C stove, ground and used to determine dry matter (DM), CP, and IAA according the procedures described by the AOAC International (2006) and Joselyn (1970). To evaluate the supplementation of crystalline amino acids on osmotic balance, the pH of diets were measured and samples of dietary treatments were sent to external laboratories for amino acids analyses.

The nitrogen balance was calculated using the equation proposed by Matterson et al. (1965), with this information we calculated the nitrogen utilization efficiency (NUE) and nitrogen utilization for gain (NWG).

At d 21, chickens and feed were weighed to evaluate feed intake, body weighed gain and feed-to-gain ratio. Broiler mortality and temperature was recorded twice daily. At d 22, the closest two birds of the average body weigh in each cage were bled via cardiac puncture for determination of plasmatic (Exp.1) and serum (Exp.2) uric acid (UA) (Donsbough et al., 2010), total protein (tP), albumin (Alb) and globulin (Glob). Broilers were fasted for two hours, and feeders were reintroduced to each pen for 30 minutes. According Donsbough et al., 2010 this UA protocol can represent the AA utilization in broilers fed AA-adequate and AA- deficient diets. Blood was placed into 10 ml heparin tubes (Exp1) and serum tubes (Exp.2) and samples were held on ice until centrifugation of 4000 rpm for 15 minutes. Afterwards, the plasma and serum was collected and separated by bird and stored in a -40°C freezer. Plasma and Serum in each experiment was analyzed for UA, tP, Alb, Glob using a commercial reagent kits (Bioclin). Using the UA and nitrogen intake it was possible to calculate the UA/g N intake and make a correlation among these data.

Each pen was considered a experimental unit for all analyses. Data was analyzed by ANOVA followed by Dunnett and SNK test ( $p < 0.05$ ) using a randomized complete design with GLM procedure of SAS Institute (2009).

## **RESULTS AND DISCUSSION**

### ***Experiment 1***

The analyzed values of CP and AAs diets were in close agreement with the calculated values, the pH was lower in treatment three supplemented with glutamic acid (Table 3). Glutamic

acid in high concentrations can decrease the pH of diet and affect the feed intake. In a parallel study we evaluated how strong is his hydrogenionic potential trough the titrate with sodium bicarbonate and potassium carbonate, and it was clear that there is a negative correlation between glutamic acid supplementation and dietary pH, that is more effectively corrected using potassium carbonate than sodium bicarbonate.

No effects of eN-to-tN ratio were observed for feed intake of broilers during the experiment (Table 4). The addition of 4.2% Glu in low CP treatments numerically reduced the feed intake. Although Glu is an excellet source of nonspecific nitrogen, the response of broilers to diets supplemented with Glu varies (Kerr and Kidd, 1999). Some authors had a negative effect on feed intake of broilers fed with Glu supplementation in diets (Ando et al., 1989; Deschepper and De Groote, 1995), while other authors did not show any effect of Glu on feed intake (Leclercq et al., 1994).

Weight gain and feed conversion ratio worsened with the reduction of CP in diet and the increased of 8% on eN-to-tN ratio. The dietary CP reduction supplemented with essential crystalline amino acids to meet AA requirements can decrease the growth rate and FCR specifically due a lack of nitrogen to synthesize non essential amino acids (Pesti et al., 2009; Dean et al., 2006; Payne, 2007). The addition of 4.2% Glu was sufficient to recover 5% the weight gain and improved in 7% the feed-to-gain ratio in low protein diets. There was no difference between positive control treatment and the negative control treatment with Glu; some researches showed the same benefits of the use of Glu in low protein diets (Moran and Stilborn, 1996; Han et al., 1992; Parr and Summers, 1991).

The nitrogen intake and nitrogen balance were higher on the PC and NC+Glu than the values for NC. The use of Glu as a source non essential nitrogen increased the levels of PUA/ g of NI compared with PC and NC. It could be associated to a higher absorption and metabolism of

crystalline amino acids compared with intact protein. There were no significant effect of nitrogen ratios on PUA, tP, Alb and Glob.

### ***Experiment 2***

The diet pH results (Table 1 and 2) showed a little difference for treatment supplemented with Glu, that tends to be more acid than others even with the addition of potassium carbonate. Although it presented lower pH it was not sufficient to limit the broilers feed intake.

The glycine supplementation as a source of non essential amino acid worsened the broiler's performance compared with other treatments, it might be caused by the amount of glycine+serine of 4.98% which resulted in 3.3g/day of glycine. Patton, 1983 in many trials observed a toxic effect of glycine in laying hens fed with 4g/day of glycine. The toxicity effect may be due for the high quantity of soluble nitrogen that overloads the kidney capacity and leads to a nephrotoxicity in high protein diets or a high free amino acids diets (Patton, 1983).

Broilers fed with 18.5% CP increased feed intake without increases in weight gain that resulted in worsened FCR compared to control treatment. Reports in the literature dealing with low CP diets have shown that keeping AA concentrations at a constant ratio to CP will not support optimal growth of broilers (Pinchasov et al., 1990; Cabel and Waldroup, 1991). The performance was recovered when it was reduced the eN-to-tN for 47.06% by the supplementation of Glu, Ala or Mix.

Nitrogen intake was higher for both of diets with 47.06% eN-to-tN, while the nitrogen balance and nitrogen utilization efficiency was lower for diets with 47.06% supplemented with non essential amino acids. Chickens fed with higher eN-to-tN ratio showed a higher nitrogen utilization efficiency per weight than other treatments. The SUA and SUA/g of NI was higher in treatments supplemented with Glu, Ala and Mix.

The objective of Exp.1 was to evaluate if low protein diets with high eN-to-tN decrease performance in broilers chicks and if the supplementation with Glu is capable to recover performance. The results show an increase in FCR, but no effect on BWG or FI when CP was reduced from 22.25% to 20.5%, even though the diet met all AA requirements of broilers. In agreement Si et al., (2004a, b) and Dean et al., 2006 reported a decrease in FCR when dietary CP levels were reduced below 22%, other researches established that performance was not reduced until 20% of CP (Sterling et al., 2005). Some experiments with poultry have been carried out to study the effect of adding NEAA to low CP diets, and the results show a positive response to high non essential nitrogen levels by the decreasing FI, moderate reduction in growth rate, and improving FCR (Heger, 2003; Parr and Summers, 1991; Han et al., 1992). Among the crystalline amino acids, glutamate seems to be the most effective source of non specific nitrogen (Allen and Baker, 1974).

The effect of non essential amino acid supplementation in low CP diets is back being the focus of recent researches, specially when it is used the ideal protein concept. The synthesis of protein involves the availability of EAA and NEAA at the context of mRNA translation, which means that in a cellular level all amino acids can be considered essential (Bedford and Summers, 1984). In the absence of NEAA, it could occur the catabolism of EAA to synthesize NEAA. Pesti et al., 2009 suggested that for the optimum WG and FCR ratio is not sufficient to have a low CP diet supplemented with essential crystalline amino acid, but also it is important to guarantee a balance between EAA and CP or EAA and NEAA. Harper and other investigators found that the absence of NEAA from chicken diets could not support maximal growth (Murayama, Sunde and Harper, 1975). Results of some researches have shown that NEAA also plays an important role in regulating gene expression, cell signaling, nutrient transport and metabolism, intestinal microbiota, anti-oxidative responses, immune responses (Wu, 2013). Deschepper et al., 1995

conducted an experiment with broiler from 7 to 42 days old in which they studied the effect of CP reduction ranging from 21 to 18% and the supplementation with Glu and Gly. Broilers fed on diets containing Glu and Gly showed similar performance as positive control.

Instead of no effect on FI reduction, Glu can regulate FI due to its low pH, playing an osmotic effect role. Glu is an important amino acid for the synthesis of glutamine and Gamma-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous system and has the function to relieve the intensity of stress. Data from animal studies suggest that decreased GABA levels in brain are associated with anxiety like behavior (Dalvi and Rodgers, 2001; Shekhar et al., 2006) while the increased GABA levels with decreased anxiety (Sherif and Orelan, 1995) in rats. In avian there is some contradictions about Glu effect on feed consumption (Zeni, 1997). Dean et al., 2006 observed a decrease on FI when broilers were fed a diet with 16.2% of CP plus Glu.

The nitrogen balance study showed a higher NI and NE for the positive control and the NC+ Glu what led to a higher nitrogen balance for these treatments. However, the NUE did not differ among treatments. Bregendahl *et al.* (2002) observed that birds fed in a low CP diets had a lower nitrogen retention than those ones fed in high CP diets. In contrast, Aletor et al. (2000) found a higher NUE when reduced CP level from 22.5% to 15.3%. No effect was observed for PUA, Alb and Glob, however, PUA/NI tends to be higher for birds fed NC+Glu, probably due to the higher amount of crystalline amino acids in diet.

In Exp.2, the addition of Gly had a negative effect on performance, probably due to a high amount of Gly+Ser in the low CP levels of 4.98%. This was not observed by other researchers that worked with Gly+Ser until 3% in low CP diets and had a linear increase on G:F ratio, it was recommended a 2.44% of Gly+Ser in diet for optimal growth of broilers (Dean et al., 2006).

Deschepper and Grooten, 1995 worked with 3.2% of sodium glutamate and 0.85% of glycine in low CP diets, showed that birds fed with NEAA had equal performance of those fed high protein.

Birds fed in a low CP levels had a higher FI and a worsened FCR than those fed in a PC and NC+NEAA. This was confirmed by Goulart (2010) that found an increase on FI as the dietary CP level reduced. This results are in agreement with those of Namroud et al. (2008) who fed birds diets as 17% of CP and saw negative effect on weight gain and FCR. Adding Gly to low CP diets with a high portion of crystalline amino acids improved performance but not completely to overcome the adverse effects of a high quantity of crystalline amino acids in low CP diets (Namroud *et al.*, 2010). In our experiment, the NEAA supplementation through Glu, Ala or a Mix were efficient to recover performance. The best FCR with 47.06% eN-to-tN was a result of lower FI, once we did not have affect on weight gain. Probably the effect on FI was a result of the lesser need of EAA utilization to synthesize NEAA, once non-essential nitrogen was supplied on diet through Glu, Ala or the Mix. Some researchers have seen the sparing effect of NEAA in the EAA utilization (Allen & Baker, 1974; Heger et al., 1998; Heger, 2003). In contrast to our results, Hussein et al. (2001) added 2.8 or 5.5% of L-glutamate in low CP diets and observed that birds fed with those diets, were not able to recover performance, suggesting that even the protein reduction from 22.6% to 17.6% non specific nitrogen was not a limitant factor.

Dean et al., 2006 evaluated different sources of NEAA for broilers fed in a low CP diets. It was used a positive control of 22.2% and a negative control of 16.2% supplemented with Gly, Glu, Ala, Asp, Pro and a Mix of all those NEAA, chicks fed diets with all NEAA had reduced FI, daily gain, and body weight compared to positive control. In the other hand, chicks fed with Ala or Glu had no difference on FCR compared to positive control. As the CP is reduced in a diet, to meet all the requirements, the amount of crystalline amino acids is increased, these authors worked with 27.1kg/ton while we had 22.8kg/ton in the negative control. The high amount of free

AAs in the diet can impact performance by changes on the nitrogen metabolism. The limitation on replacing intact protein with crystalline amino acids could be partly due to increased blood ammonia and uric acid (Namroud et al., 2008; Maia et al., 2017).

Glycine is essential for feather development, representing 15% of feather protein and 30% of the AA present in collagen and it is an important component of the uric acid molecule. However, in the present study there is no doubt that the high inclusion of glycine can lead to a toxic effect for broilers. The reduction on NI on birds fed NC+Gly can be associated to the limitation on feed intake, with this an increase on NB and NUE.

Birds fed with NC + Glu, NC+ Ala or NC+ Mix had no differences on NI compared to the PC, however they had a higher NE and a lower NB than PC. This difference is caused by the different apport of nitrogen in the diet; free amino acids are faster metabolized than nitrogen on intact protein. It become clearer when comparing the SUA and SUA/NI for treatments supplemented with NEAAs. They had higher production of UA per g of nitrogen intake. The NC showed a better NWG than other treatments because there was no effect on body weight when eN:tN ratio increased.

In conclusion, it is very important to understand the effect of NEAA role on low crude protein diets, although they are classified as non- essential, they are involved in a lot of metabolic pathways. Low crude protein diets has a higher eN-to-tN ratio that probably lead to a deficiency on non-specific nitrogen, the addition of NEAA to reach the same eN-to-tN ratio as a practical diet, could be an important step to apply with greater assertiveness the concept of ideal protein. Broilers fed low protein diets and a higher eN-to-tN, even meeting all amino acids requirements, show worst performance compared with a high crude protein diet. The use of Glu, Ala or a Mix (Glu, Ala, Gly) in low protein diets is able to recover performance at the same level as feeding a high protein diets.

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**Table 1.** Composition of basal diets for chicks in experiment 1.

<b>Treatments</b>			
<i>eN:tN (%)</i>	<b>47.66</b>	<b>51.48</b>	<b>47.66(+Glu)</b>
<b>Ingredients</b>	<b>Amount (%)</b>		
Corn	45.58	51.63	51.63
Soybean Meal	37.28	31.50	31.50
Corn Gluten Meal	2.00	2.00	2.00
Soybean Oil	3.40	2.37	2.37
Dicalcium Phosphate	1.516	1.565	1.565
Limestone	0.884	0.883	0.883
Salt	0.400	0.200	0.200
Sodium bicarbonate	0.127	0.421	0.421
L-Lisine HCl (79%)	0.141	0.315	0.315
DL-Methionine (99%)	0.252	0.300	0.300
L-Treonine (98%)	0.017	0.094	0.094
L-Valine (96.5%)	0.000	0.060	0.060
L-Arginine (98.5%)	0.000	0.057	0.057
L-Glycine (99%)	0.000	0.202	0.202
L-Isoleucine (99%)	0.000	0.011	0.011
Premix. <sup>1</sup>	0.395	0.395	0.395
Insoluble Acid Ash (IAA)	1.000	1.000	1.000
L- Glutamic Acid (56.80% CP)	0.000	0.000	2.800
Corn Starch	7.000	7.000	4.200
<b>Chemical Composition</b>			
Crude protein, analyzed%	21.90	20.43	22.49
ME, kcal/kg	3.075	3.075	3.075
Calcium, %	0.819	0.819	0.819
Available phosphorus, %	0.391	0.391	0.391
Sodium, %	0.210	0.210	0.210
Dig Lysine, %	1.174	1.174	1.174
Dig Met + Cys, %	0.846	0.846	0.846
Dig Threonine, %	0.763	0.763	0.763
Dig Tryptophan, %	0.245	0.214	0.214
Dig Arginine, %	1.374	1.268	1.268
Dig Valine, %	0.939	0.904	0.904
Dig Isoleucine, %	0.873	0.787	0.787
Dig Gly + Ser, %	1.838	1.838	1.838
Dig Histidine, %	0.537	0.485	0.485
pH	7.42	6.79	6.61

<sup>1</sup>Mineral premix (amount per kg diet): manganese - 77.0 mg, iron - 55.0 mg, zinc - 71.5 mg, copper - 11.0 mg, iodine - 1.10 mg, and excipient q.s. - 1,000 g; Vitamin premix (amount per kg diet): vitamin A - 8250 IU, vitamin D3 - 2090 IU, vitamin E - 31.0 IU, vitamin B1 - 2.20 mg, vitamin B6 - 3.08 mg, pantothenic acid - 11.0 mg, biotin - 0.077 mg, vitamin K3 - 1.65 mg, folic acid - 0.77 mg, nicotinic acid - 33.0 mg, vitamin B12 - 0.013 mg, selenium - 0.33 mg, and excipient q.s. - 1,000 g); 1 g choline chloride 60%/kg diet; 0.1 g butylated hydroxytoluene/kg diet; 0.55 g sodium salinomycin 12m/kg diet; 0.1 g avilamycin 10mg/kg diet.

**Table 2.** Composition of basal diets for chicks in experiment 2.

<b>Treatments</b>						
<i>eN:tN (%)</i>	<b>47.06</b>	<b>56.95</b>	<b>47.06 (+Gly)</b>	<b>47.06 (+Ala)</b>	<b>47.06 (+Glu)</b>	<b>47.06 (+Mix<sup>1</sup>)</b>
<b>Ingredients</b>	<i>Amount (%)</i>					
Corn	42.63	48.90	48.90	48.90	48.90	48.90
Soybean Meal	38.43	25.04	25.04	25.04	25.04	25.04
Corn Gluten Meal	2.00	2.00	2.00	2.00	2.00	2.00
Soybean Oil	4.26	1.956	1.956	1.956	1.956	1.956
Dicalcium Phosphate	1.512	1.651	1.651	1.651	1.651	1.651
Limestone	0.881	0.871	0.871	0.871	0.871	0.871
Salt	0.420	0.151	0.151	0.151	0.151	0.151
Sodium bicarbonate	0.100	0.500	0.500	0.500	0.500	0.500
Potassium carbonate	0.000	0.300	0.300	0.300	0.540	0.300
L-Lisine HCl (79%)	0.111	0.532	0.532	0.532	0.532	0.532
DL-Methionine (99%)	0.247	0.383	0.383	0.383	0.383	0.383
L-Treonine (98%)	0.006	0.205	0.205	0.205	0.205	0.205
L-Valine (96.5%)	0.000	0.203	0.203	0.203	0.203	0.203
L-Glycine (99%)	0.000	0.486	0.486	0.486	0.486	0.486
L-Arginine (98.5%)	0.000	0.274	0.274	0.274	0.274	0.274
L-Isoleucine (99%)	0.000	0.142	0.142	0.142	0.142	0.142
L - Histidine HCl (74%)	0.000	0.038	0.038	0.038	0.038	0.038
L-Tryptophan (99%)	0.000	0.025	0.025	0.025	0.025	0.025
Premix. <sup>2</sup>	0.395	0.395	0.395	0.395	0.395	0.395
Insoluble acid ash	1.000	1.000	1.000	1.000	1.000	1.000
L-Glycine	0.000	0.000	3.486	0.000	0.000	2.040
L-Alanine	0.000	0.000	0.000	4.041	0.000	1.224
L – Glutamic Acid	0.000	0.000	0.000	0.000	7.287	0.816
Corn Starch	8.000	14.941	11.455	10.900	7.654	10.861
<b>Chemical Composition</b>						
Crude protein, analyzed %	21.09	18.99	22.70	21.40	21.30	21.90
ME, kcal/kg	3.075	3.075	3.075	3.075	3.075	3.075
Calcium, %	0.819	0.819	0.819	0.819	0.819	0.819
Available phosphorus, %	0.391	0.391	0.391	0.391	0.391	0.391
Dig Lysine, %	1.174	1.174	1.174	1.174	1.174	1.174
Dig Met + Cys, %	0.846	0.846	0.846	0.846	0.846	0.846
Dig Threonine, %	0.763	0.763	0.763	0.763	0.763	0.763
Dig Tryptophan, %	0.250	0.200	0.200	0.200	0.200	0.200
Dig Arginine, %	1.401	1.268	1.268	1.268	1.268	1.268
Dig Valine, %	0.951	0.904	0.904	0.904	0.904	0.904
Dig Isoleucine, %	0.888	0.787	0.787	0.787	0.787	0.787
Dig Gly + Ser, %	1.866	1.838	4.982	1.838	1.838	3.680
Dig Histidine, %	0.543	0.435	0.435	0.435	0.435	0.435
Ph	6.65	7.50	7.74	7.80	5.58	6.89

<sup>1</sup> 50% L-Glycine, 30% Alanine, 20% Glutamate; <sup>2</sup>Mineral premix (amount per kg diet): manganese - 77.0 mg, iron - 55.0 mg, zinc - 71.5 mg, copper - 11.0 mg, iodine - 1.10 mg, and excipient q.s. - 1,000 g; Vitamin premix (amount per kg diet): vitamin A - 8250 IU, vitamin D3 - 2090 IU, vitamin E - 31.0 IU, vitamin B1 - 2.20 mg, vitamin B6 - 3.08 mg, pantothenic acid - 11.0 mg, biotin - 0.077 mg, vitamin K3 - 1.65 mg, folic acid - 0.77 mg, nicotinic acid - 33.0 mg, vitamin B12 - 0.013 mg, selenium - 0.33 mg, and excipient q.s. - 1,000 g); 1 g choline chloride 60%/kg diet; 0.1 g butylated hydroxytoluene/kg diet; 0.55 g sodium salinomycin 12mg/kg diet; 0.1 g avilamycin 10mg/kg diet.

**Table 3.** Exp.1 Effect of Glu supplementation and eN to tN ratio on performance and blood parameters of broilers chick<sup>1</sup>.

Treatments	Ne:Nt (%)	WG(kg)	FI (kg)	FCR	PUA(mg/dL) <sup>‡</sup>	tP(mg/dL)	Glob(mg/dL)	Albu(mg/dL)
T1 - 22.25% CP	47.66	0.824A	1.124	1.365A	3.01	2.904	1.560	1.508
T2 - 20.5% CP	51.48	0.775B*	1.115	1.444B*	2.96	3.166	1.560	1.606
T3 - 20.5%+Glu	47.66	0.812A	1.097	1.351A	3.93	3.098	1.537	1.561
P-Value		0,0222	0.4248	0,0133	0.0510	0.2129	0.2351	0.4121
SEM		0,0341	0,0211	0,0224	0.1862	0.0626	0.0422	0.0295

<sup>‡</sup>Plasmatic uric acid.

\*Dunnett Test response (P < 0.05).

<sup>A,B</sup> SNK response (P<0.05)

<sup>1</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011).

**Table 4.** Exp.1 Effect of Glu supplementation and eN to tN ratio on nitrogen utilization parameters of broilers chick<sup>1</sup>.

Treatments	Ne:Nt (%)	NI (g/kg)	NE(g/bird)	NB(g/bird)	NUE(g/bird)	NWG(g/bird)	PUANg <sup>‡</sup> (g/bird)
T1 - 22.25% CP	47.66	46.48A	19.06	27.42A	58.98	17.88	0.0645
T2 - 20.5% CP	51.48	41.89B*	16.89	25.00B*	59.66	18.51	0.0708
T3 - 20.5%+Glu	47.66	45.00A	17.65	27.35A	60.74	18.05	0.0875
P-Value		<0.001	<0.001	<0.001	0.7543	0.2288	0.0575
SEM		0.4815	0.3491	0.2236	0.2061	0.1557	0.0041

<sup>‡</sup>Plasmatic uric acid per g of nitrogen intake.

\*Dunnett Test response (P < 0.05).

<sup>A,B</sup> SNK response (P<0.05)

<sup>1</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011).

**Table 5.** Exp.2 Effect of Gly, Ala and Glu supplementation and eN-to-tN ratio on performance and blood parameters of broilers chick<sup>1</sup>.

Treatments	Ne:Nt (%)	FBW (kg)	WG(kg)	FI (kg)	FCR	SUA(mg/dL) <sup>‡</sup>	tP	Glob	Albu
T1 - 22.25% CP	47.06	1,065A	0,859A	1,165BC	1,357BC	7.31B	2.959	1.281	1.678
T2 - 18.5% CP	56.95	1,071A	0,861A	1,243A*	1,445D*	7.07B	2.818	1.290	1.528
T3 - 18.5% CP + Gly	47.06	0,923B	0,716B	0,928D	1,297A	7.50B	2.703	1.143	1.560
T4 - 18.5% CP + Glu	47.06	1,067A	0,860A	1,182B	1,375B	9.81A	2.897	1.241	1.656
T5 - 18.5% CP + Ala	47.06	1,044A	0,837A	1,128C	1,347BC	10.31A	2.984	1.330	1.654
T6 - 18.5% CP + Gly+Ala+Glu	47.06	1,065A	0,858A	1,134C	1,322AB	11.84A	2.980	1.337	1.643
P-Value		<0,001	<0,001	<0,001	<0,001	<0.001	0.4622	0.3036	0.4140
CV(%)		0.044	0.065	0.013	0.002	0.3390	0.0456	0.0257	0.0244

<sup>‡</sup> Serum uric acid.

\*Dunnett Test response (P < 0.05).

<sup>A,B</sup> SNK response (P<0.05)

<sup>1</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011).

**Table 6.** Exp.2 Effect of Gly, Ala and Glu supplementation and eN-to-tN ratio on nitrogen utilization parameters of broilers chick<sup>1</sup> from 8 to 21 days.

Treatments	Ne:Nt (%)	NI (g/kg)	NE(g/bird)	NB(g/bird)	NUE(g/bird)	NWG (g/bird)	SUANg <sup>‡</sup>
T1 - 22.25% CP	47.06	45.54A	19.55A	25.99A	57.10B	18.87B	0.159C
T2 - 18.5% CP	56.95	43.30B*	18.75A	24.55B*	56.71B	19.88A*	0.163C
T3 - 18.5% CP + Gly	47.06	38.17C*	12.30B*	25.87A	68.01A	18.77B	0.195BC
T4 - 18.5% CP + Glu	47.06	45.70A	22.74C*	22.96C*	50.27C	18.83B	0.215AB
T5 - 18.5% CP + Ala	47.06	44.13AB	21.10C*	23.03C*	52.36C	18.98B	0.233AB
T6 - 18.5% CP + Gly+Ala+Glu	47.06	45.07A	21.65C*	23.42C*	51.92C	19.05B	0.263A
P-Value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
CV(%)		0.3794	0.044	0.2095	0.9168	0.0883	0.0075

<sup>‡</sup>Serum uric acid per g of nitrogen intake..

\*Dunnett Test response (P < 0.05%).

<sup>A,B</sup> SNK response (P<0.05%)

<sup>1</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011).

ESSENTIAL TO TOTAL NITROGEN RATIO AND USE OF NON-ESSENTIAL AMINO ACIDS IN  
POULTRY DIETS

**Essential-to-total nitrogen dietary ratios using a mixture of non-essential amino acid on  
performance, nitrogen metabolism and blood parameters for broilers**

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Section: Metabolism and Nutrition

**ABSTRACT** Three-hundred male Cobb<sup>®</sup> 500 from 8 to 21d were weighed and allocated to a completely randomized experimental design with ten replicate pens, each one receiving six dietary treatments. The treatments consisted of a positive control with 22,25%CP and 47% of essential-to-total nitrogen ratio (eN-to-tN), and other five treatments with different eN-to-tN ratio (56%, 53%, 50%, 47% and 44%) using a basal diet with 19%CP supplemented with graded levels of a mixture of Glu+Ala+Gly (60:20:20). Diets were formulated using corn, gluten corn, soybean meal 46% and 1% of IAA. From 14d to 21d, excretas were collected by indicator methodology, to enable the calculation of nitrogen utilization. On d 22 chickens were bled via cardiac puncture, to quantify uric acid, total protein, albumin and globulin. It was evaluated the variables of nitrogen utilization, performance and blood parameters. Data were analyzed by Dunnett test and the growth performance and blood parameters of the five progressive levels data were analyzed using requirement-estimation models as a linear response plateau (LRP; i.e., piecewise regression) and quadratic broken line (QBL). Birds fed diets with 56% (eN-to-tN) showed lower body weight gain (BWG) at 21d, lower feed intake (FI) and worst feed conversion rate (FCR) than birds fed diets with 47% of eN-to-tN. Nitrogen utilization efficiency (NUE) and nitrogen utilization efficiency for gain (NUEG) were higher for treatments with 56%, 53% and 50%. QBL effect was observed just for serum uric acid (SUA), the performance parameters showed a LRP effects with the Xplat very close to 50% of eN-to-tN. The reduction on protein diets, without loose broiler performance, should be done until meet the eN-to-tN ratio of 50%.

**Keywords:** nitrogen, non-essential amino acids, protein.

**Abbreviations:** AA, amino acids; EAA, essential amino acids; NEAA, non-essential amino acids; N, nitrogen; eN, essential nitrogen; tN, total nitrogen; NEN, non-essential nitrogen

## INTRODUCTION

Protein has been classified as the most expensive nutrient in diets for non-ruminants, therefore a lot of researches were done as a tool to reduce the crude protein levels and consequently the feeding costs. Protein reduction is associated not only in feeding cost but also in decreasing the nitrogen excretion and the environmental pollution (Kerr and Easter, 1995; Rotz, 2004). The excess of protein can lead to higher amount of amino acids in the gut and in the protein synthesis site, while once met the amino acids requirement, their excess will be catabolized what spends a lot of energy on excretion.

The large use of industrial amino acids in diets allow higher reduction in protein levels. Once established that animals absorb amino acids, it is acceptable that if we compare diets with high protein and low protein plus crystalline amino acids to meet the animal requirement, they should show the same performance response, but it does not occur in practice (Summer et al., 1991). It happens because although they have the same amount of essential amino acids (EAA) for growth, they have not the same amount of non-essential amino acids (NEAA) and consequently a different amino acid ratio.

Currently there are a lot of disparity among data in literature for estimated EAA-to-NEAA ratio, it is probably due to the different ways to express the classification of EAA and NEAA and methodological approaches (Heger, 2003).

The AA ratios can be expressed in protein base, amino acid base, and in nitrogen base. Due to the range difference in nitrogen source content of NEAA, diets can have the same amount of NEAA sum and different N concentrations. Since the main role of NEAA is working as a non-specific nitrogen, the best way to relate amino acids in diet is expressing them in the nitrogen molecular weight.

To express nitrogen ratios, it is possible to relate essential nitrogen (eN) to non-essential nitrogen (NeN), or eN to total nitrogen (tN). The second one is more practical because if NeN tend to zero the ratio eN-to-NeN goes to infinite (Heger, 2003).

The definition of eN could be done by the sum of nitrogen of total essential amino acids in diet or in animal requirement. When we use the requirement to express the essential nitrogen it seems to be closer of biological reality, this because all the excess of essential amino acids will be catabolized and used as a source of nitrogen for the animal.

In practical diets we have high crude protein levels that leads in low eN-to-tN ratio, as we reduce the crude protein, we also decrease tN in diets that leads a high eN-to-tN ratio. The optimum ratio about eN and tN is important to understand how much can we reduce protein without see decrease on performance. This concept is very important because ideal protein should be more than essential amino acids ratio with lysine but also a ratio between essential and non-essential amino acid.

Based on this information, two experiment were carried out to evaluate essential to total nitrogen ratio supplemented with NEAA in low protein diets and their effects on performance, nitrogen retention and blood parameters of broiler chickens from 8 to 21 d post hatch.

## **MATERIALS AND METHODS**

The experimental procedures were previously supervised by the Ethics Committee on Animal Use and conducted according to the experimental protocol for use of live birds (Brazilian College of Animal Experimentation). It was carried out on Poultry Farm at Federal University of Viçosa. Chickens were raised until 8d of age on masonry house divided into circles protection with wood-shavings litter, tube feeders and manual drinkers with *ad libitum* access to food and water. The lighting program consisted of 24L and temperature was kept close to 32°C in the first

week and gradually reduced according the Cobb<sup>®</sup> manual recommendations. A corn and soybean meal pre-starter diet was formulated to meet all the nutrients recommendation proposed by Rostagno *et al.*, 2011. On d 8 birds were weighed and randomly allocated to six dietary treatments placed into 10 replicates with five birds each. A positive control was used to contain nutrients levels as commercial diet with low eN:tN ratios. A high eN-to-tN dietary ratio was formulated using corn starch, corn gluten meal, corn, soybean meal and crystalline amino acids to meet nutritional recommendations except for protein (Rostagno *et al.*, 2011). The other treatments were obtained by the addition of a non-essential amino acids mixture with alanine, glycine and glutamic acid (60:20:20). The treatments were: T1 = Control treatment with 22.25% CP (47% eN-to-tN); T2 = 19.0% CP (56% eN-to-tN); T3 = 19.0%CP +1.201% of Mixture (53% eN-to-tN); T4 = 19.0% CP + 2.516% of Mixture(50% eN-to-tN); T5 = 19.0% CP + 3.999% of Mixture (47% eN-to-tN);T6 = 19.0% CP + 5.683% of Mixture (44% eN-to-tN) (Table 1). The nitrogen ratio was calculated in all diets considering the requirement of essential amino acids multiplied by their nitrogen content. The total nitrogen was calculated through the sum of nitrogen content in the diet feedstuffs composition. All diets were supplemented with 1% of insoluble acid ash (IAA) to measure the nitrogen excretion and calculate the nitrogen balance, nitrogen utilization efficiency (NUE) and nitrogen utilization for gain (NWG). To maintain the same amount of dietary electrolyte balance (DEB, close to 200mEq/kg) and pH in diets, it was used potassium carbonate and sodium bicarbonate.

From 14d to 21d, excretas were collected twice daily to measure nitrogen content, dry matter and IAA according the procedures described by the AOAC International (2006) and Joselyn (1970). The nitrogen balance was calculated using the equation proposed by Matterson *et al.*, (1965) cited by Sakomura and Rostagno (2016). Growth performance were measure at d 21, chickens and feed were weighed to evaluate the final body weight (FBW), feed intake(FI), body

weighed gain(BWG) and feed conversion ratio (FCR). Broiler mortality and temperature was recorded twice daily. At d 22, the closest two birds of the mean weigh in each cage were bled via cardiac puncture for determination of serum uric acid (SUA) (Donsbough et al., 2010), total protein (tP), albumin (Alb) and globulin (Glob). Before the blood collect, broilers were fasted for two hours, and feeders were reintroduced to each pen for 30 minutes. According Donsbough et al., 2010 this UA protocol can represent the AA utilization in broilers fed AA-adequate and AA-deficient diets. Blood was placed into 10 ml serum tubes and samples were held on ice until centrifugation of 4000 rpm for 15 minutes. The serum was collected , separated by bird and stored in a -40°C freezer for analysis of SUA, tP, Alb, Glob using a commercial reagent kits (Bioclin). With the results of SUA and nitrogen intake we calculated the SUA/g N intake (SUANg).

All data were initially subjected to a 1-way ANOVA using the PROC GLM procedure of SAS/STAT software (version 9.2; SAS Institute, 2009) to be significant with alpha = 0.05. Subsequently, it was used a Dunnett test to compare the positive control and other treatments, for the five quantitative treatments, each response parameter was modeled using only linear and non linear regression analyses. A linear response plateau (**LRP**) model was used to determine the optimal eN-to-tN ratio for each evaluated parameter (Pesti et al, 2009), and a quadratic broken-line (**QBL**) regression was computed for each response using the NLIN procedure (Robbins et al., 2006).

## RESULTS AND DISCUSSION

The eN-to-tN ratio affected directly broiler performance. Birds fed diets with 56%( eN-to-tN) showed lower body weight gain(BWG) at 21d, lower feed intake(FI) and worst feed conversion rate(FCR) than birds fed diets with 47% of eN-to-tN from intact protein.

There was not difference on performance of broilers fed diets with eN-to-tN of 50%, 47% and 44%, while birds fed with 53% showed worst feed conversion ratio than birds fed with control treatment. (Table 2). The eN-to-tN ratio did not have a quadratic or quadratic broken line effect on performance parameters, whereas a linear response plateau effects were observed on final body weight (FBW) as shown by the equation  $FBW = 1.3118 - 0.0054X_i$  ;  $X_i > 50.0%$  ;  $R^2 = 0.91$ ; BWG as shown by the equation  $BWG = 1.128 - 0.0054X_i$  ;  $X_i > 50.0%$  ;  $R^2 = 0.93$ , FI as shown by the equation  $FI = 0.7351 + 0.0079X_i$  ;  $X_i > 48.4%$  ;  $R^2 = 0.69$  and FCR as shown by the equation  $FCR = 0.6021 + 0.0145X_i$  ;  $X_i > 48.3$  ;  $R^2 = 0.72$ . For blood parameters results, only uric acid showed difference when it was used different eN-to-tN ratio. A Dunnett test showed difference when it was compared the uric acid concentration of the control treatment 47% with treatments of 47% and 44% plus mixture. A linear and a quadratic response plateau had a significant effect on UA (mg/dL) shown by the equation UA (LRP) =  $39.94 - 0.696X_i$  ;  $X_i < 50.3%$ ;  $R^2 = 0.98$  and UA (QBL) =  $120.19 - 4.199X_i + 0.0382X_i^2$  ;  $X_i < 54.9%$ ;  $R^2 = 0.96$ .

Dietary CP levels is inversely proportional of eN-to-tN ratio, higher nitrogen ratio means lower CP levels. It was clearly that when CP levels was reduced, the broilers decrease performance even with the apport of EAAs. This could be done by the lack caused of the apport of non-specific nitrogen used to synthesize NEAAs. When the mixture of glutamic acid, glycine and alanine was added the performance was recovered until the plateau.

Summers, Spratt and Atinkson 1991 showed that birds fed diets with similar EAA level, but varying widely in level of NEAA, consumed similar amounts of feed and deposited similar amounts of carcass protein. Some researchers have reported that birds fed low crude protein diets had worst performance than birds fed higher protein diets, although when glutamate was used as a source of NEAA it failed to improve growth performance, suggesting

that the low crude protein diet was not limiting on non-essential nitrogen (Hussen, Cantor and Pescatore, 2001; Lecqlerq et al., 1994). Probably it occurred due the high reduction in protein levels done by athours of 5,3% that could limited the amount of EAA.

Moran and Stilborn et al., 1996 reported that addition of Glu to low CP diets given from 0 to 3wk of age improved BW gain of broiler chicks. They suggested that improvement in BW gain occurred when Glu supplementation was equivalent from 1 to 2% CP. At the same context, Fancher and Jensen, 1989 reported that addition of Glu to some low CP diets with supplementation of EAA improved growth performance of broiler chicks from 3 to 6 wk of age, compared with high CP control diet.

Parr and Summers, 1991 found that supplementing the low crude protein diets with NEAA in the form of Ala, Asp, and Glu failed to improve performance of broiler chicks from 7 to 21d of age. Their results suggest that the low CP diets were not enough deficient in nonessential N compounds. In birds fed high CP diets, NEAA can be synthesized from excess of EAA, although the conversion efficiencies is different from each amino acid. In contrast, when birds are fed low CP diets with minimal levels of EAA, synthesis of NEAA from excess of EAA would be reduced. The utilization of L-Lysine HCl and L-Arginine as a source of non-specific nitrogen for growing chicks relative to L-Glu is about 50% and 36% respectively (Allen and Baker, 1974).

Food intake by animals is affected by some factors as corn granulometry, energy levels, quality of dietary protein and by dietary AA. When we refer specifically for AA and proteins, food consumption by animals could be depressed in response to a severe deficiency of dietary protein or an individual AA (particulary an EAA or conditional EAA), a distortion of the dietary pattern of AA when protein intake is either high or low and a substantial increase in dietary protein or AA content. Under all those conditions, changes in feeding behavior are associated

with substantial alterations in concentrations of many AA especially those that are the precursors for the synthesis of neurotransmitters (Wu, 2013).

Alanine, glycine and glutamic acid seems to be a good combination of NEAA as a source of non specific nitrogen. The major site production of glutamate and glutamine is in the avian skeletal muscle and hepatocytes from the catabolism of branched chain amino acids (BCAAs). BCAAs donate an amino group to  $\alpha$ -ketoglutarate to form glutamate, which is either amidated to produce glutamine or transaminated with pyruvate to yield alanine. Glutamine and Alanine are two major source of nitrogen and carbon in the interorgan metabolism of AA (Goldberg and Chang, 1978), and they have an important role as a energy source for cells of immune system and defending the host against infectious disease.

Albumin and globulin was measured to evaluate if the different apport of nitrogen would affect the immune capacity of broiler. It could be better measured if the broilers were submitted to immune challenge, that did not occurred in this experiment.

Uric acid is synthesized in liver and kidney, and excreted by tubular secretion. It is an important parameter to understand kidney disorders, the increase in SUA is associated with some deseas or imbalance of AAs in diet, an increase until 15mg/dL is observed in severe kidney disorders caused by vitamin A deficiency, mycotoxins and overdoses apport of protein in diets. In this study, the serum uric acid results showed an important step of crystalline amino acids metabolism. Diets with higher apport of protein from crystalline amino acids have higher absoption rate than intact protein diets that lead an increase of metabolism rate with higher serum uric acid production. Although 44% , 47% and 50% of eN-to-tN ratio showed a recover on performance, 44% and 47% had a significant higher production of uric acid.

Nitrogen excretion and nitrogen intake had a linear response as increase the nitrogen in diet, although the nitrogen balance did shown a linear response. The efficiencie of nitrogen

utilization for gain was higher for diet with 56% of eN-to-tN and had a linear positive correlation with the nitrogen ratios in diet. Serum uric acid per g of nitrogen showed that the crystalline amino acids supplementation increase the use of nitrogen to synthesize uric acid. In the results of SUA it was shown that broilers receiving diets with 44 and 47% of eN-to-tN plus mixture produce more SUA than the control treatment with intact protein, but when it was measured the production per g of nitrogen intake it was clear that the addition of the mixture had a linear effect for uric acid production. These results are important to understand how the metabolism of nitrogen can be regulated with the use of crystalline amino acids without affect the performance parameters.

In conclusion, there is a limit to reduce protein in broilers diets and the nitrogen ratio should be considered when diets are formulated using the ideal protein concept. The lack of non specific nitrogen leads to increase the catabolism of essential amino acid to provide the necessary amount of nitrogen to synthesize non-essential amino acids. To keep broiler performance similar to broilers fed high protein diets, low-CP diets should not have more than 50% of eN-to-tN ratio.

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**Table 1.** Ingredients and analyzed nutrient composition of the basal diet.

<b>Treatments</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
	CP	22.25%	19%	19% + Mix	19% + Mix	19% + Mix
<i>Ingredients</i>	Ne:Nt (%)	47	56	53	50	47
		22.25%	19%	19% + Mix	19% + Mix	19% + Mix
Corn		44.61	56.88	56.88	56.88	56.88
Soybean Meal 45%		38.10	26.96	26.96	26.96	26.96
Corn Gluten Meal (60%)		2.00	1.00	1.00	1.00	1.00
Soybean Oil		4.61	2.73	2.73	2.73	2.73
Bicalcium Phosphate		1.51	1.61	1.61	1.61	1.61
Limestone		0.88	0.88	0.88	0.88	0.88
Salt		0.42	0.15	0.15	0.15	0.15
Sodium Bicarbonate		0.100	0.500	0.500	0.500	0.500
Potassium Carbonate		0.000	0.122	0.122	0.122	0.122
L-Lysine HCl (79%)		0.116	0.461	0.461	0.461	0.461
DL-Methionine (99%)		0.245	0.360	0.360	0.360	0.360
L-Threonine (98%)		0.006	0.172	0.172	0.172	0.172
L-Valine(96,5%)		0.000	0.164	0.164	0.164	0.164
L-Glycine (99%)		0.000	0.283	0.283	0.283	0.283
L-Arginine (98,5%)		0.000	0.204	0.204	0.204	0.204
L-Isoleucine(99%)		0.000	0.110	0.110	0.110	0.110
L-Tryptophan (99%)		0.000	0.013	0.013	0.013	0.013
L - Histidine HCl (74%)		0.000	0.002	0.002	0.002	0.002
Premix <sup>2</sup>		0.395	0.395	0.395	0.395	0.395
IAA		1.000	1.000	1.000	1.000	1.000
Corn Starch		6.000	6.000	4.799	3.484	2.001
Mixture (15.45%N) <sup>1</sup>		-	-	1.201	2.516	3.999
<b>Chemical Composition</b>						
Crude protein, analyzed %		22.60	18.32	19.48	20.76	22.18
ME, kcal/kg		3.075	3.075	3.075	3.075	3.075
Calcium, %		0.819	0.819	0.819	0.819	0.819
Available phosphorus, %		0.391	0.391	0.391	0.391	0.391
Sodium, %		0.210	0.210	0.210	0.210	0.210
Lysine, dig%		1.174	1.174	1.174	1.174	1.174
Met+Cis, dig%		0.846	0.846	0.846	0.846	0.846
Threonine, dig%		0.763	0.763	0.763	0.763	0.763
Glutamate, analyzed %		3.162	2.775	3.518	3.666	3.815
Glycine, analyzed %		0.942	1.062	1.355	1.448	1.540
Alanine, analyzed %		0.969	1.439	2.061	2.567	2.910
DEB (mEq/kg)		204.13	203.88	203.88	203.88	203.88
pH		6.14	6.90	6.73	6.45	6.26

<sup>1</sup> 60% Alanine, 20% L-Glycine, 20% Glutamate; <sup>1</sup> 1.1g Mineral Premix (amount per kg diet): manganese - 77.0 mg, iron - 55.0 mg, zinc - 71.5 mg, copper - 11.0 mg, iodine - 1.10 mg, and excipient q.s. - 1,000 g; 1.1g Vitamin premix (amount per kg diet): vitamin A - 8250 IU, vitamin D3 - 2090 IU, vitamin E - 31.0 IU, vitamin B1 - 2.20 mg, vitamin B6 - 3.08 mg, pantothenic acid - 11.0 mg, biotin - 0.077 mg, vitamin K3 - 1.65 mg, folic acid - 0.77 mg, nicotinic acid - 33.0 mg, vitamin B12 - 0.013 mg, selenium - 0.33 mg, and excipient q.s. - 1,000 g; 1 g choline chloride 60%/kg diet; 0.1 g butylated hydroxytoluene/kg diet; 0.55 g sodium salinomycin 12mg/kg diet; 0.1 g avilamycin 10mg/kg diet.

**Table 2.** Effect of eN-to-tN ratio on growth performance and blood parameters during the starter phase<sup>1</sup>.

Treatments	Ne:Nt (%)	FBW (kg)	WG(kg)	FI (kg)	FCR	SUA(mg/dL)	tP	Glob	Albu
T1 - 22.25% CP (control)	47.00	1.049	0.861	1.132	1.312	5.47	2.95	1.36	1.44
T2 - 19.0% CP	56.0	1.012*	0.828 <sup>‡</sup>	1.164	1.394*	4.49	3.03	1.40	1.47
T3 - 19.0% CP + 1.20% Mix <sup>2</sup>	53.0	1.020	0.835	1.178	1.409*	5.32	2.98	1.56	1.43
T4 - 19.0% CP + 2.51% Mix	50.0	1.046	0.861	1.117	1.307	5.07	2.96	1.48	1.49
T5 - 19.0% CP + 3.99% Mix	47.0	1.039	0.856	1.106	1.293	7.31*	3.14	1.45	1.53
T6 - 19.0% CP + 5.68% Mix	44.0	1.046	0.860	1.128	1.312	9.25*	3.08	1.44	1.49
Dunnett's test		0.0482	0.0521	0.1886	0.004	<0.001	ns	ns	ns
<i>Regression Analysis</i>									
Linear		0.001	0.001	0.010	0.013	0.001	ns	ns	ns
Quadratic		ns	ns	ns	ns	0.001	ns	ns	ns
Linear Broken Line (LBL)		0.068	0.001	0.015	0.034	0.001	ns	ns	ns
Quadratic Broken Line (QBL)		ns	ns	ns	ns	0.001	ns	ns	ns
CV		2.99	3.55	6.23	5.06	34.5	11.1	12.3	8.53

\*Dunnett Test response (P < 0.05) vs Control T1

<sup>‡</sup> Dunnett Test response (P < 0.10).

<sup>1</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011).

<sup>2</sup> Mixture of Ala:Glu:Gly (60:20:20).

**Table 3.** Effect of eN-to-tN ratio on content of N in excreta, N intake (NI), N balance, nitrogen utilization efficiency (NUE), nitrogen utilization for gain (NWG) and serum uric acid per g of NI (SUAN) on chickens during the starter phase<sup>1</sup>.

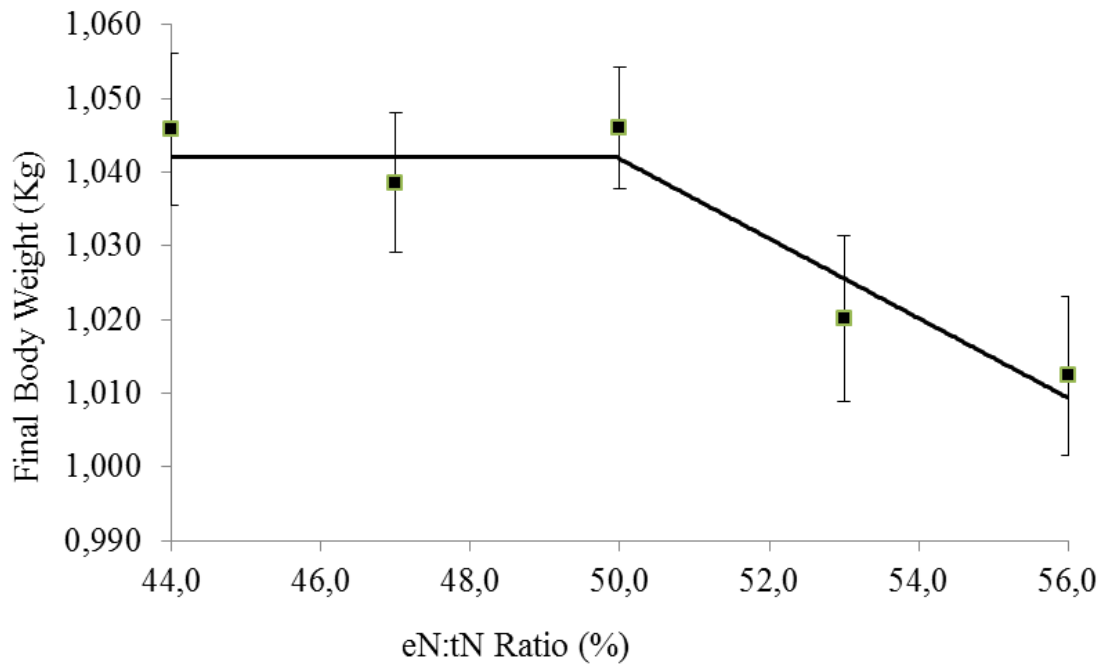
Treatments	Ne:Nt (%)	NE <sup>2</sup> (g/kg)	NI(g/kg)	BN(g/bird)	NUE (%)	NWG(g)	SUAN(g)
T1 - 22.25% CP (control)	47.00	52.03	46.50	23.16	49.92	18.59	0,118
T2 - 19.0% CP	56.0	39.38*	38.77*	23.58	61.63*	21.42*	0,117
T3 - 19.0% CP + 1.20% Mix <sup>2</sup>	53.0	44.41*	41.72*	24.29	57.83*	20.13*	0,120
T4 - 19.0% CP + 2.51% Mix	50.0	48.93	42.15*	23.86	56.83*	20.50*	0,128
T5 - 19.0% CP + 3.99% Mix	47.0	60.33*	44.58	25.10*	54.49*	19.20	0.164
T6 - 19.0% CP + 5.68% Mix	44.0	65.53*	48.82	25.19*	52.19	17.64	0,191
Dunnett's test		<0.001	<0.001	<0.001	<0.001	<0.001	0.3608
<i>Regression Analysis</i>							
Linear		<0.001	<0.001	<0,001	<0.001	<0.001	<0.001
Quadratic		0.2592	0.1417	ns	0.0766	ns	ns
CV(%)		8.07	6.10	4.15	7.93	5.29	37.92

\*Dunnett Test response (P < 0.05).

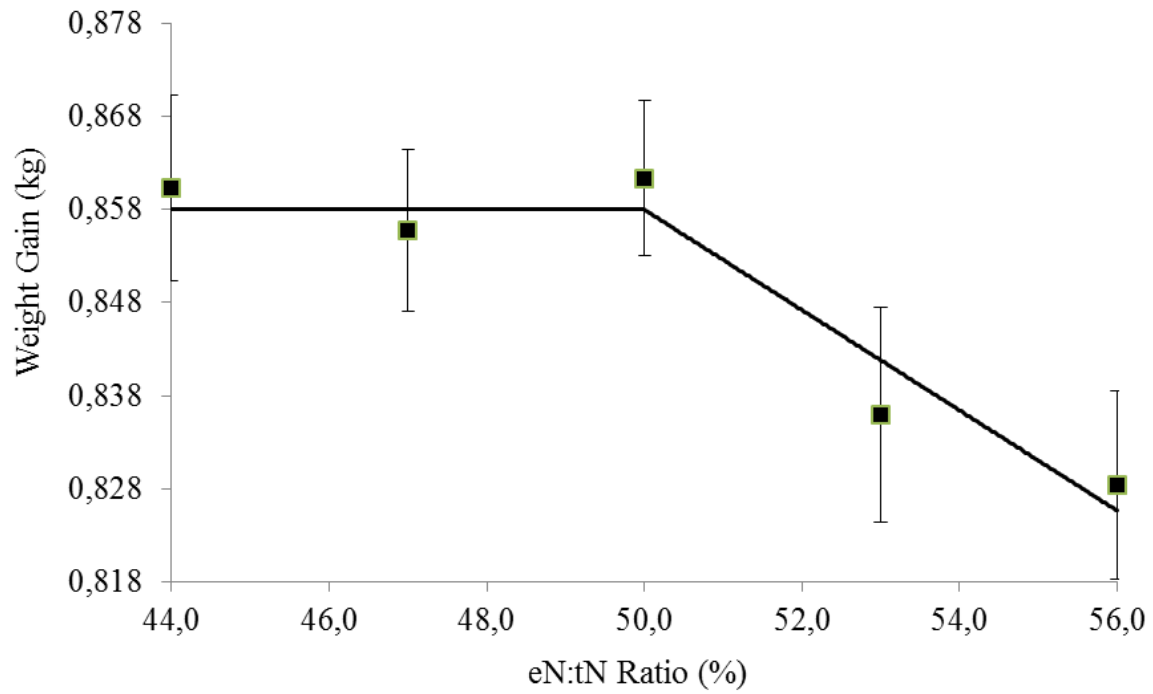
<sup>1</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011).

<sup>2</sup>Content of nitrogen in the excreta (dry matter basis);

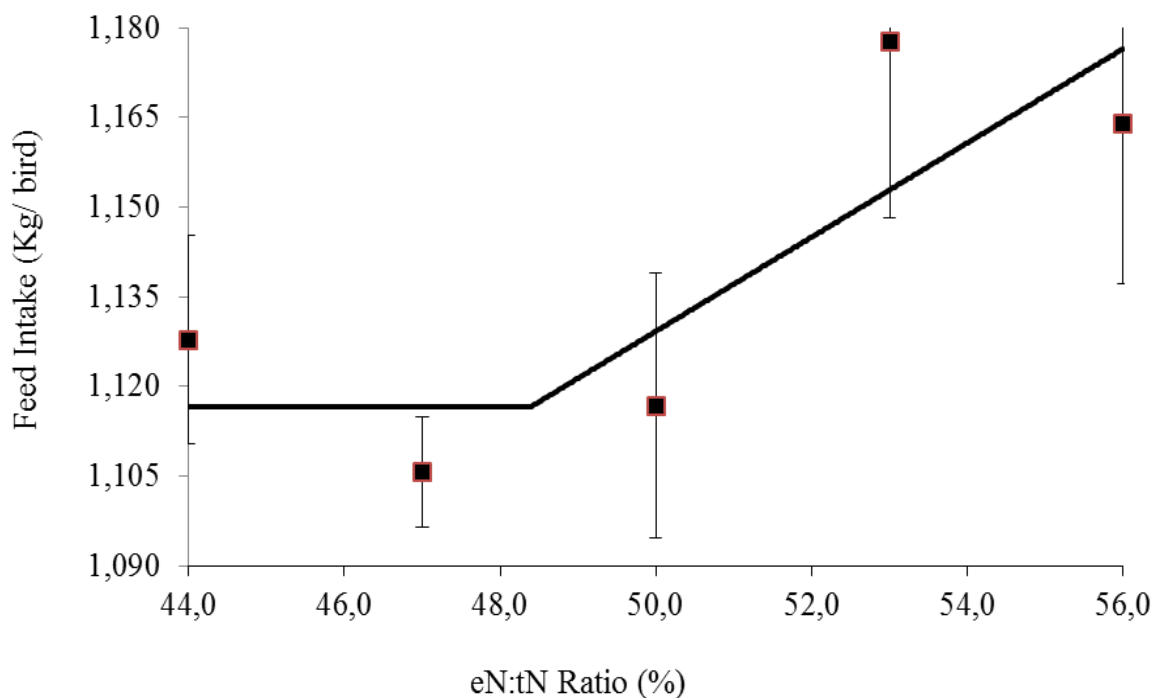
<sup>3</sup> Mixture of Ala:Glu:Gly (60:20:20).

**Figure 1**

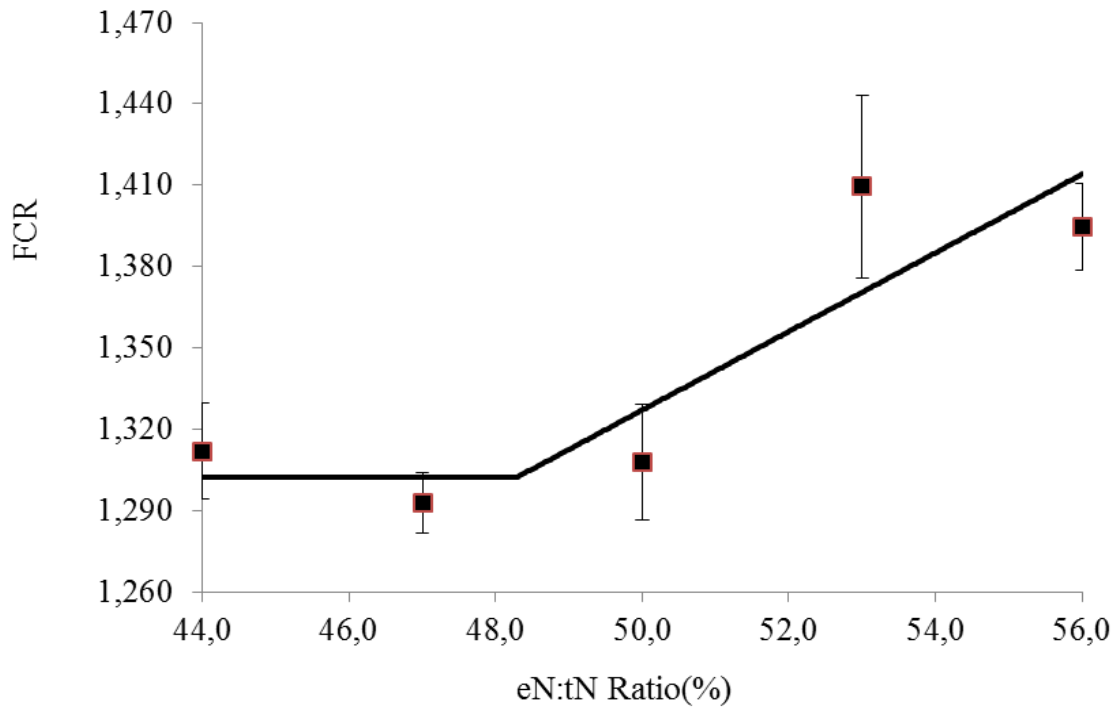
**Figure 1.** Modeled effects of dietary eN-to-tN ratios on final body weight gain of broiler chicks in the feeding period from 8 to 21 d of age. Using the LRP model, an optimal ratio of 50% was estimated, with a plateau response of 1.042 kg and an increasing slope to that plateau ( $y = 1.3118 - 0.0054X_i$ ;  $R^2 = 0.91$ ).

**Figure 2**

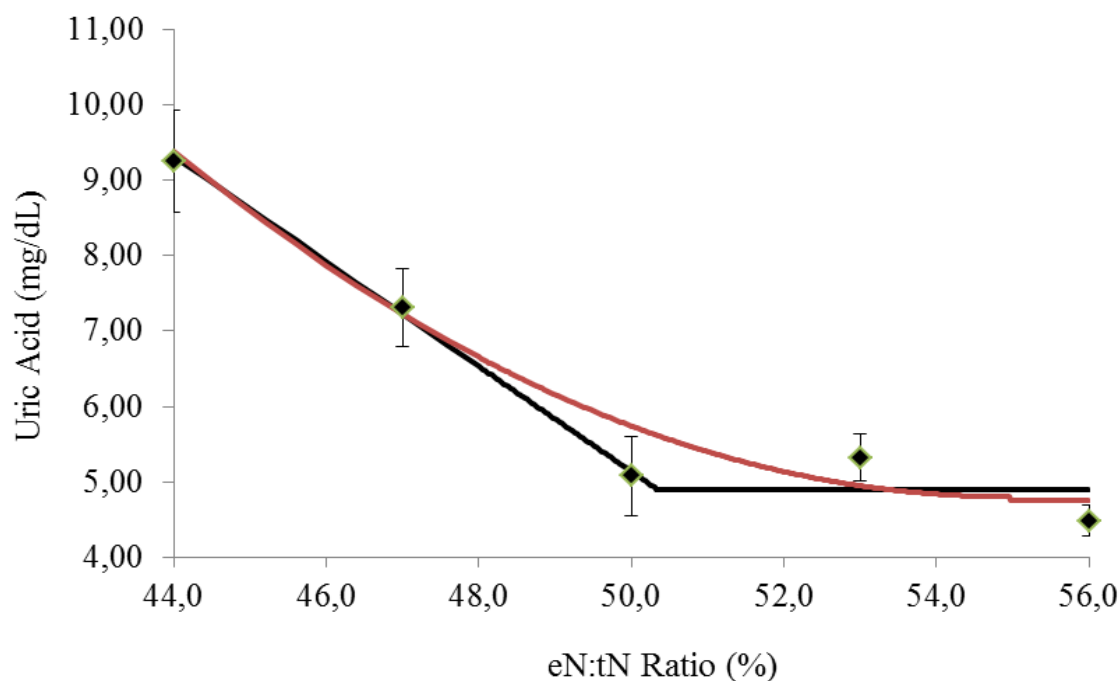
**Figure 2.** Modeled effects of dietary eN-to-tN ratios on feed intake of broiler chicks in the feeding period from 8 to 21 d of age. Using the LRP model, an optimal ratio of 50% was estimated, with a plateau response of 0.858 kg and an increasing slope to that plateau ( $y = 1.128 - 0.0054Xi$ ;  $R^2 = 0.92$ ).

**Figure 3**

**Figure 3.** Modeled effects of dietary eN-to-tN ratios on weight gain of broiler chicks in the feeding period from 8 to 21 d of age. Using the LRP model, an optimal ratio of 48.4% was estimated, with a plateau response of 1.116 kg and an increasing slope to that plateau ( $y = 0.7351 + 0.0079X_i$ ;  $R^2 = 0.69$ ).

**Figure 4**

**Figure 4.** Modeled effects of dietary eN-to-tN ratios on feed conversion ratio of broiler chicks in the feeding period from 8 to 21 d of age. Using the LRP model, an optimal ratio of 48.28% was estimated, with a plateau response of 1.302 kg and an increasing slope to that plateau ( $y = 0.6021 + 0.0145X_i$ ;  $R^2 = 0.79$ ).

**Figure 5**

**Figure 5.** Modeled effects of dietary eN-to-tN ratios on serum uric acid (mg/dL) of broiler chicks in the feeding period from 8 to 17 d of age. Using the LRP model, an optimal ratio of 50.3% was estimated, with a plateau response of 4.93 mg/dL and an increasing slope to that plateau ( $y = 39.94 - 0.696Xi$ ,  $r^2 = 0.98$ ). The QBL model was also significant ( $P < 0.01$ ) with a plateau response of 4.80 mg/dL [ $y = 120.19 - 4.199Xi + 0.0382Xi$ , if  $Xi < 54.9$ ; otherwise, SUA (mg/dL) = 4.80 ( $P < 0.01$ ,  $r^2 = 0.96$ ).

**Table 4.** Summary of estimated optimal eN-totN ratios for broiler chicks in the starter phase.

Response	Model	Equation	Plateau	R <sup>2</sup>	eN:tN(%)
FBW	LRP	$Y = 1.3118 - 0.0054X_i$	1.0420	0.91	50.00
FI	LRP	$Y = 0.7351 + 0.0079X_i$	1.1162	0.69	48.40
BWG	LRP	$Y = 1.128 - 0.0054X_i$	0.8581	0.92	50.00
FCR	LRP	$Y = 0.6021 + 0.0145X_i$	1.3022	0.79	48.28
SUA	LRP	$Y = 39.94 - 0.696X_i$	4.9300	0.98	50.30
SUA	QBL	$Y = 120.19 - 4.199X_i + 0.0382X_i^2$	4.8000	0.96	54.90
NE	L	$Y = -2.2742X_i + 165.42$	-	0.97	-
NI	L	$Y = -0.7656X_i + 81.49$	-	0.93	-
BN	L	$Y = -0.1342X_i + 31.11$	-	0.77	-
NUE	L	$Y = 0.6739X_i + 23.29$	-	0.89	-
NUEG	L	$Y = 0.283X_i + 5.628$	-	0.87	-
SUANg	L	$Y = -0.006X_i + 0.462$	-	0.88	-

<sup>1</sup>Optimal eN-to-tN ratio estimates shown for each response in which at least one model was significant. Abbreviations: L, linear; Q, quadratic, LRP-to-quadratic regression ratio; QBL, quadratic broken line.

## SOURCES OF NITROGEN FOR BROILERS FED LOW PROTEIN DIETS

**Evaluation of nitrogen source in low crude protein diets on performance and blood parameters for broilers chickens**

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Section: Metabolism and Nutrition

**ABSTRACT** A study was carried out to evaluate the supplementation of glutamic acid (Glu) and urea in low-CP diets and their effects on performance and blood parameters of broiler chickens from 8 to 21 d age. A total of 200 eight-day-old Cobb® 500 male broiler were distributed in a completely randomized 2 x 2 factorial arrangement for a total of 4 treatments with 10 replicates of 5 birds each. The treatments consisted of a low CP basal diet with 19%CP and 56% of eN-to-tN, Glu and urea was used to compound other treatments and meet a 50% of eN-to-tN ratio. The treatment were as follow: T1, 19%CP and 56% of eN-to-tN; T2, T1 +Glu; T3, T1+urea; and T4, T1+Glu+urea. Growth performance were measure at d 21, and on d 22, birds were bled via cardiac puncture for determination of serum uric acid (SUA), total protein (tP), albumin (Alb) and globulin (Glob). Diets were analyzed for nitrogen, to enable the calculation of the nitrogen efficiency utilization for gain (NUEG) and the serum uric acid production per g of nitrogen intake (SUANg). All data were subjected to a 1-way ANOVA using the PROC GLM (SAS 9.2) and compared by a Dunnett test. No effects of Glu or urea supplementation were observed on final body weight (FBW), weight gain (WG), feed intake (FI), total protein (tP), globulin (GLOB), albumin (ALBU), and SUANg. However, the Glu supplementation showed a significant improvement of 5% on feed conversion rate (FCR). When urea was individually supplemented to low protein diet it failed to improve performance, but the combination of Glu and urea was efficient to improve FCR in 3% more than control treatment. The NUEG was lower when urea was individually added to a low-CP diet, it proves the certain difficulty of chicken to use a non protein nitrogen source. In conclusion, urea should not be used alone in diets to reduce 6% of eN-to-tN ratio, but the supplementation with Glu or the combination of Glu and urea seems to be a good tool to improve performance of chickens fed a low-CP diets.

**Keywords:** nitrogen, essential amino acids, poultry.

**Abbreviations:** AA, amino acids; EAA, essential amino acids; NEAA, non-essential amino acids; N, nitrogen; eN, essential nitrogen; tN, total nitrogen; NEN, non-essential nitrogen

## INTRODUCTION

The large use of industrial amino acids in diet allows higher reduction in protein levels. Once established that animals absorb amino acids, it is acceptable that if compared diets with high protein and low protein plus crystalline amino acids to meet the requirement, they should show the same performance response, but it does not occur in practical. Protein value has much more functions in the body beyond amino acids source. Currently diets are formulated to meet crude protein recommendations, but do the protein values is represented by just amino acids? Actually most of nitrogen analysis are done by Kjeldahl or Dumas methodologies that consist in measure nitrogen content in general. Nitrogen content is multiply for a conversion factor (6.25) to arrive at protein content. This factor is an average of earlier determination and means that the content of nitrogen in protein represent 16% (FAO, 2003).

The conversion factor of 6.25 has been argued because the nitrogen content in protein will change follow the amino acid profile in protein. The nitrogen content of an specific amino acid change according molecular weight and number of nitrogen atoms it contains. As an example, arginine is an essential amino acid with 32% of nitrogen presented in 0.37% and 3.35% in corn and soybean meal respectively (Rostagno et al., 2017), therefore it should not be applied the same conversion factor for all feedstuffs in diet. Because this Jones (1931) suggested that  $N \times 6,25$  should be abandoned and replaced by  $N \times$  specific conversion factor for each ingredient in the diet. For many years this specific factors were known as “Jones factors”, and currently other authors have been studied the amount of nitrogen in different ingredient and their specific factors (Sriperm. Pesti, and Tillman, 2011).

Some researches are necessary to understand how can we meet exactaly the amount of protein required for broilers. The use of ideal protein is an alternative to reduce the excess of nitrogen in diets and consequently the nitrogen excretion. But some studies have shown that birds

fed a reducing protein and adding crystalline amino acids did not present same results from those fed with a high protein diet. It could be explained by the reduction of non-specific nitrogen to synthesize non essential amino acid (NEAA). The supplementation of NEAAs as glutamic acid seems to be a good strategy to work as a nitrogen source and recover broiler performance (Maia *et al.*, 2016). The last advance on ideal protein concept is to determine the amino acids or nitrogen ratio in diets, for this is necessary to understand how the nitrogen is utilized and until what level it is possible to reduce protein, reduce feed costs without loose performance.

A lot is known about the utilization of protein nitrogen by poultry but there are still a lack of information of non protein nitrogen utilization. Urea, diammonium citrate and diammonium phosphate are some of the sources studied in the past decades, but no researches about their combination with protein nitrogen and their effects on performance and blood parameters.

In this context it was carried out a study to evaluate the effects of the association of glutamic acid and urea on performance and blood parameters for broilers

## **MATERIALS AND METHODS**

The experiment was carried out on Poultry Farm at Federal University of Viçosa. Chickens were raised until 8d of age on masonry house divided into circles protection with wood-shavings litter, tube feeders and manual drinkers with *ad libitum* access to food and water. A corn and soybean meal pre-starter diet was formulated to meet all the nutrients recommendation proposed by Rostagno *et al.*, 2011. On d 8, 200 birds were weighed and randomly allotted to four dietary treatments, in factorial arrangement 2x2, placed into 10 replicates with five birds each. Feed was provided in mash form, and birds had free access to feed and water. The experimental diets were as follows: T1, a low CP diet based on corn and soybean meal formulated with 19% CP and 56% eN-to-tN ratio; T2 = T1 added L-Glutamic Acid; T3 = T1 added urea; and T4 = T1

added L-Glutamic Acid and urea. The L-Glutamic Acid and urea supplementation was provide to each treatment until it meet 50% eN to tN. Corn starch was used in low CP diets and replaced to L-Glutamic Acid or urea to compose other treatments.

The nitrogen ratio was calculated in all diets considering the requirement from total essential amino acids multiplied by their nitrogen content. The total nitrogen was calculated through the sum of nitrogen content in the diet feedstuffs composition. The apport of nitrogen in the treatment with Glu and urea was calculated to provide half of the amount of nitrogen in the protein form and non protein form. To maintain the same amount of dietary electrolyte balance (DEB, close to 200mEq/kg) and pH in diets, it was used potassium carbonate and sodium bicarbonate.

Growth performance were measure at d 21, chickens and feed were weighed to evaluate the final body weight, feed intake, body weighed gain and feed-to-gain ratio. Broiler mortality and temperature was recorded twice daily. At d 22, the closest two birds of the mean weigh in each cage were bled via cardiac puncture for determination of serum uric acid (SUA) (Donsbough et al., 2010), total protein (tP), albumin (Alb) and globulin (Glob). Before the blood collect, broilers were fasted for two hours, and feeders were reintroduced to each pen for 30 minutes. According Donsbough et al., 2010 this UA protocol can represent the AA utilization in broilers fed AA-adequate and AA- deficient diets. Blood was placed into 10 ml serum tubes and samples were held on ice until centrifugation of 4000 rpm for 15 minutes. The serum was collected , separated by bird and stored in a -40°C freezer for analysis of SUA, tP, Alb, Glob using a commercial reagent kits (Bioclin).

It was analyzed nitrogen content in diets to calculate the nitrogen utilization for gain (NWG) and the serum uric acid per g of nitrogen intake (SUANg) as follow:

$$gNWG = WG(g)/NI(g)$$

$$SUANg: SUA(mg/dL) / NI(g)$$

WG = Weight gain (g/bird);

NI = Nitrogen intake (g/bird);

SUA = Serum uric acid (g/bird);

### *Statistical Analysis*

All data were initially subjected to a one-way ANOVA using the PROC GLM procedure of SAS software (version 9.2; SAS Institute, 2009) to be significant with  $\alpha = 0.05$ . Subsequently, it was used a Dunnett test to compare the treatment with 19 %CP and other treatments.

## **RESULTS AND DISCUSSION**

The urea supplementation in low CP diets promoted higher value of pH (7.22) than low CP diets (5.94). The addition of Glu in that treatment was sufficient to reduce pH for 5.5. Commercial diet with 22% of CP formulated with corn and soybean meal tends to present pH close to 7, and as it was reduced CP and supplemented with crystalline amino acids the pH is also decreased.

No effects of eN-to-tN ratio were observed on final body weight(FBW), weight gain (WG), feed intake (FI), total protein (tP), globulin (GLOB), albumin (ALBU), and serum uric acid per g of nitrogen intake (SUANg). However, the Glu supplementation showed a significant improvement of 5% on feed conversion rate (FCR). Maia *et al.*, 2014 also found that the use of Glu to decrease the eN-to-tN ratio in low protein diets was effective to improve FCR. It occurs probably due a lack of non-specific nitrogen in higher eN-to-tN for the synthesis of non-essential

amino acid. Berres *et al.*, (2010) suggested that it is vital to provide non-essential nitrogen to birds that are fed low CP, particularly in the early growth stages, as NEAA can become limiting below a certain level of dietary protein. Han *et al.*, 1992 observed that chicks fed a low CP diet fortified with five limiting AA and Glu gained at the same rate with similar feed efficiency and had estimated body fat levels comparable to birds fed the 23% CP.

When urea was individually supplemented to low protein diet it failed to improve performance, but the combination of Glu and urea was efficient to improve FCR in 3%.

The use of non protein nitrogen(NPN) in monogastric diets is questionable, urea is still unconventional in poultry feeding unlike ruminant animals, it has long been considered as toxic to poultry but some researches has yielded controversial results. Although caecum is found to be the major site of ammonia production from urea, the called nutritional benefit is probably limited to its utilization for the synthesis of non-essential amino acids in low protein diets (Chowdhury, Roy and Sarker, 1996). Sullivan and Bird (1937) were able to show that nitrogen from urea and diammonium citrate (DAC) could be utilized better by chicks fed in diets with methionine hydroxy analogue. Featherston *et al.* (1962) and Shannon (1970) using highly purified crystalline amino acid diets demonstrated significant use by the chick of mixtures L- and D-essential amino acids, mixtures of dispensable amino acids, diammonium citrate and urea.

The capacity of the use on NPN by birds is related with the environment, intestinal microflora, age and other factors. Okomura *et al.*, (1976) observed that the germ free chicks did not benefit from urea supplementation whereas conventional birds showed improved feed conversion efficiency and significantly better growth were reported. They stated that the gut microorganism are responsible for the growth promoting effect of urea, presumably through the release of NH<sub>3</sub>, by bacterial urease and its consequence incorporation into amino acids. In our

study to provide the better utilization of NPN, the dietary nitrogen was limited to half and half from protein and non-protein nitrogen.

Birnbaum *et al.* (1957) found that diets in which L-alanine, ammonium L-glutamate, L-glutamine, ammonium L-aspartate and L-proline were individually furnished as the sources of dispensable nitrogen supported most rapid growth. Other authors found that L-glutamic acid and a mixture of L-indispensable amino acids were the most effective supplements to recover growth compared to low protein diets (Rehcgil *et al.*, 1957).

Featherston, Bird and Harper, 1962, feeding birds with urea and ammonium citrate found an increase on nitrogen retention and increased plasma levels of dispensable amino acids. The authors indicated that the nitrogen from urea and ammonium citrate was retained and converted to non-essential amino acid, but when the levels of essential amino acids of the basal diet were increased, urea was not as effective as the non-essential amino acids. The authors found a worsened NWG for birds fed low CP+urea compared to a low CP + non-essential amino acids or a high CP diet. This found corroborates with the decrease on NWG observed in this study, there was a significant difference of 6.6% between 56% eN-to-tN and a 50% eN-to-tN adding urea concluding that NPN alone worsened the efficiency of weight gain *per g* of nitrogen intake. It occurred due the higher amount of nitrogen intake for the same amount of weight gain, however no statistic difference was found for feed intake.

Although there was no statistic difference in SUA between treatments, the addition of Glu tends to increase the SUA . The Glu supplementation increase in 14% the uric acid in serum of birds, whereas Urea decrease in 4%. This evidence contribute with the theory of higher absorption and metabolism of free amino acids specially when it is used Glu. There are two main process involved in Glu absorption, a mediated route with high apparent affinity and a diffusion mechanism (Lerner and Steinke, 1976), both of them shows no tendency to saturate in the

millimolar concentration of Glu in the chicken intestine. The absorption of some amino acids are influenced by the amount of other amino acids, but some studies suggest that a distinct transport system exists for acidic amino acids in the chicken intestine, and Glu has little effect on basic or neutral amino acid transport (Lerner and Steinke, 1976).

The serum uric acid *per g* of NI showed that the supplementation of Glu increases in 14% the SUANg, whereas urea supplementation decrease in 10% SUANg. It was not find in the literature reseaches compared the SUANg for NPN. The results can be associated with the different metabolic pathway for NPN and PN. The main site of NEAA absorption in the jejunum, while for urea is in the ceacum after microorganism fermentation to produce NH<sub>3</sub>. The NH<sub>3</sub> can be absorbed by the large intestine wall and used for NEAA synthesis.

The eN-to-tN ratio has an important role in ideal protein concept as a toll to explain how far can we reduce protein levels without decrease performance. However, it is well established that NEAA supplementation is able to recover performance of birds fed very low CP diet, but the use of NPN should be evaluated more closely. As we found in our results, adding all non specific nitrogen at the urea form, it failed to recover FCR, but when it was added Glu and urea the FCR was improved. Probably due the better utilization of Glu by chicks.

In conclusion, urea can partially be used by chicken as a non-specific nitrogen source to meet a 50% of eN-to-tN ratio, since part of nitrogen be added by Glu supplementation. Whereas individually Glu supplementation seems to be a good way to improve performance on birds fed a low CP diets.

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**Table 1.** Ingredients and analyzed nutrient composition of the basal diet

<b>Treatments</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	
	CP	19%	19% + Glu	19% +Urea	19% +Glu+Urea
<b>Ingredients</b>	Ne:Nt (%)	56	50	50	50
Corn		62.41	62.41	62.41	62.41
Soybean Meal 45%		24.09	24.09	24.09	24.09
Corn Gluten Meal (60%)		2.00	2.00	2.00	2.00
Soybean Oil		1.54	1.54	1.54	1.54
Bicalcium Phosphate		1.61	1.61	1.61	1.61
Limestone		0.88	0.88	0.88	0.88
Salt		0.14	0.14	0.14	0.14
Sodium Bicarbonate		0.500	0.500	0.500	0.500
Potassium Carbonate		0.120	0.318	0.120	0.120
L-Lysine HCl (79%)		0.530	0.530	0.530	0.530
DL-Methionine (99%)		0.354	0.354	0.354	0.354
L-Threonine (98%)		0.183	0.183	0.183	0.183
L-Valine(96,5%)		0.176	0.176	0.176	0.176
L-Glycine (99%)		0.324	0.324	0.324	0.324
L-Arginine (98,5%)		0.258	0.258	0.258	0.258
L-Isoleucine(99%)		0.128	0.128	0.128	0.128
L-Tryptophan (99%)		0.024	0.024	0.024	0.024
L - Histidine HCl (74%)		0.014	0.014	0.014	0.014
Premix <sup>2</sup>		0.445	0.445	0.445	0.445
Corn Starch		4.300	0.000	3.432	1.729
Glutamic Acid (9,09% N)		-	4.300	0.000	2.139
Urea (45,00% N)		-	0.000	0.868	0.432
<b>Chemical Composition</b>					
Crude protein, analyzed %		19.98	21.03	21.28	21.18
ME, kcal/kg		3.075	3.075	3.075	3.075
Calcium, %		0.819	0.819	0.819	0.819
Available phosphorus, %		0.391	0.391	0.391	0.391
Sodium, %		0.210	0.210	0.210	0.210
Lysine, dig %		1.174	1.174	1.174	1.174
Met+Cis, dig %		0.846	0.846	0.846	0.846
Threonine, dig %		0.763	0.763	0.763	0.763
Glutamate, analyzed %		4.150	7.440	3.200	6.280
Glycine, analyzed %		0.970	1.080	1.070	1.360
Alanine, analyzed %		1.190	1.020	0.980	1.230
DEB (mEq/kg)		199.50	199.50	199.50	199.50
pH		5.94	5.23	7.22	5.50

<sup>1</sup>1.25g Mineral Premix (amount per kg diet): manganese - 77.0 mg, iron - 55.0 mg, zinc - 71.5 mg, copper - 11.0 mg, iodine - 1.10 mg, and excipient q.s. - 1,000 g; 1.25g Vitamin premix (amount per kg diet): vitamin A - 8250 IU, vitamin D3 - 2090 IU, vitamin E - 31.0 IU, vitamin B1 - 2.20 mg, vitamin B6 - 3.08 mg, pantothenic acid - 11.0 mg, biotin - 0.077 mg, vitamin K3 - 1.65 mg, folic acid - 0.77 mg, nicotinic acid - 33.0 mg, vitamin B12 - 0.013 mg, selenium - 0.33 mg, and excipient q.s. - 1,000 g); 1.2 g choline chloride 60%/kg diet; 0.1 g butylated hydroxytoluene/kg diet; 0.55 g sodium salinomycin 12mg/kg diet; 0.1 g avilamycin 10mg/kg diet.

**Table 2.** Effect of Glu and urea supplementation on growth performance and blood parameters during the starter phase.

Treatments	Ne:Nt (%)	FBW (kg)	WG(kg)	FI (kg)	FCR	SUA(mg/dL)	tP	Glob	Albu
T1 - 19.00% CP	56.00	0.956	0.753	1.019	1.354	5.56	2.79	1.52	1.41
T2 - 19.0% CP + Glu	50.00	0.949	0.754	0.974	1.293*	6.97	2.86	1.41	1.45
T3 - 19.0% CP + Urea	50.00	0.953	0.751	1.001	1.333	5.92	2.92	1.46	1.45
T4 - 19.0% CP + Glu+Urea	50.00	0.942	0.739	0.972	1.316*	6.20	2.73	1.53	1.36
Nitrogen Source									
Glu	-	0.954	0.752	1.010	1.343b	5.74	2.86	1.49	1.43
Glu	+	0.945	0.746	0.973	1.304a	6.58	2.79	1.47	1.41
Urea	-	0.953	0.754	0.997	1.323	6.27	2.82	1.46	1.43
Urea	+	0.948	0.745	0.987	1.324	6.06	2.83	1.49	1.40
P-Value									
Glu		ns	ns	ns	0.0095	0.0647	0.2139	ns	ns
Urea		ns	ns	ns	ns	0.6462	ns	ns	ns
Glu*Urea		ns	ns	0.1850	ns	0.2144	0.0688	0.1104	0.1251
CV(%)		3.57	5.76	5.57	4.52	20.57	8.06	15.93	8.41

\*Dunnnett Test response (P < 0.05).

<sup>2</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011). In this experiment, Glu and/or urea was added replacing corn starch to produce the desired eN-to-tN ratio of 50%.

**Table 3.** Effect of Glu and urea supplementation on nitrogen intake, efficiency of nitrogen for gain and serum uric acid *per g* of nitrogen of broilers chick.

Treatments	Ne:Nt (%)	NI (g/bird)	NWG(g/bird)	SUANg
T1 - 19.00% CP	56.00	40.96	18.50a	0.149
T2 - 19.0% CP + Glu	50.00	41.17	18.28	0.172
T3 - 19.0% CP + Urea	50.00	43.07*	17.30b*	0.138
T4 - 19.0% CP + Glu+Urea	50.00	41.86	17.88	0.153
Nitrogen Source				
Glu	-	42.01	17.90	0.143
Glu	+	41.51	18.08	0.163
Urea	-	41.06	18.39	0.160
Urea	+	42.47	17.59	0.145
P-Value				
Glu		ns	0.3203	ns
Urea		0.0175	0.0020	ns
Glu*Urea		ns	0.0391	ns
CV(%)		3.38	2.57	21.18

\*Dunnett Test response (P < 0.05).

<sup>2</sup>All birds received a common pre-starter diet from day 1 to 7 post-hatch, and all diets (pre-starter and starter) met or exceeded nutrient recommendations for each age of chicks (Rostagno et al, 2011). In this experiment, Glu and/or urea was added replacing corn starch to produce the desired eN-to-tN ratio of 50%

## CONCLUSÃO GERAL

A redução proteica nas dietas é uma prática comum que visa reduzir custo e perdas de nitrogênio na excreta. Entretanto, a adoção da redução proteica deve ser feita de forma criteriosa, isso porque altas reduções podem provocar um desvio na utilização de aminoácidos essenciais para síntese de aminoácidos não essenciais. Portanto, é importante determinar a relação existente entre nitrogênio ou aminoácidos da dieta.

A suplementação de fontes de nitrogênio provindas da suplementação de aminoácidos não essenciais em dietas de baixo teor proteico se mostrou eficiente em recuperar o desempenho em frangos de corte de 8 a 21 dias de idade.

Deve-se atentar para reduções de proteína na dieta que elevem a relação de em:tN acima de 50%.

De maneira geral as fontes proteicas de nitrogênio apresentam uma boa utilização pelas aves ao passo que o nitrogênio não proteico na forma de ureia não apresentou melhoria do resultado de desempenho.