ABSTRACT

This study aimed to evaluate the effect of different reductions in digestible amino acids content (lysine, methionine, and threonine), according to two nutritional requirements in corn, soybean meal, and meat and bone meal based diets, with protease supplementation, on performance parameters and carcass characteristics. A total of 1080 day-old chicks, male, Cobb 500, were allotted to a completely randomized design, in a factorial arrangement 3 x 2, three reductions in digestible amino acids content (lysine, methionine, and threonine) and two nutritional requirements (Rostagno et al. 2005 and Cobb-Vantress Guidelines 2008), and all diets were supplemented with protease (200 ppm) with 6 replicates of 30 birds per pen. There was no significant interaction (p<0.05) between digestible amino acid reductions and both nutritional requirements for the performance variables and carcass yield and cuts. There was an effect of amino acid reduction and protease supplementation only on slaughter weight (p<0.05). Broilers fed according to the nutritional requirements of Rostagno et al. (2005) showed better (p<0.05) performance when compared to broilers fed as specified by the nutritional requirements of Cobb-Vantress (2008) with no significant differences in carcass characteristics. Protease supplementation of corn, soybean meal, and meat and bone meal based diets allows a reduction in the inclusion of crystalline amino acids (lysine, methionine, and threonine).

INTRODUCTION

Animal nutrition knowledge has been one of the factors responsible for driving productivity rates in the broiler industry. In Brazil, poultry feed is based on corn, soybean meal and meat bone meal. However, it is well known that the presence of antinutritional factors as trypsin inhibitors, lecithins and allergenic proteins in soybean meal (Rocha et al., 2014); non-starch polysaccharides and phytate in corn (Cowieson et al., 2010), and high variability of nutrient and energy composition in meat and bone meal (Troni et al., 2016), which can affect the nutritional value, utilization and digestibility of these feed ingredients, causing digestive losses to the broilers as well as reducing its performance.

Protease supplementation in diets for broilers is a relevant tool to improve the efficiency in utilizing vegetable and animal protein (Stefanello et al., 2016; Vieira et al., 2016; Cowieson et al., 2018). Protease promotes a higher degradation of antinutritional factors present in feedstuffs improves protein digestibility and decreases synthesis of endogenous enzymes, resulting in higher availability of amino acids for protein deposition (Angel et al., 2011; Kamel et al., 2015).

In order to meet broiler’s requirements, different nutritional recommendations can be used in association with enzymes added to
diets (Oliveira et al., 2012). Crude protein and digestible lysine levels are the variables with highest variation among nutritional plans, as they are among the most expensive components and an excess or deficiency will lead to amino acid imbalance. Therefore, formulation based on the ideal protein concept is able to provide a better balance in the ratio between digestible amino acids (methionine + cystine, lysine and threonine) and crude protein, thus increasing poultry productivity (Wu, 2014).

Choosing an appropriate nutritional recommendation with the inclusion of protease in the diet can lead to an increase in the availability of amino acids, once, overestimated the true amino acid digestibility of ingredients in diets supplemented with protease can be an alternative to reduce the inclusion of crystalline amino acids, consequently, reducing feed costs.

Thus, the objective of the present study was to evaluate the effect of different reductions in the amounts of digestible amino acids (lysine, methionine, and threonine), and two nutritional recommendations in corn, soybean meal, and meat and bone meal based diets, with protease supplementation on broilers performance and carcass characteristics.

**MATERIAL AND METHODS**

The study was performed according to ethical principles for animal experimentation established by the Brazilian College of Animal Experimentation (Cobea, 2009).

**Table 1** – Composition of experimental diets for each rearing period according to two nutritional recommendations, NR1 (Rostagno et al., 2005) and NR2 (Cobb-Vanterss, 2008).

| Ingredients                   | Starter | Groover | Finisher |
|-------------------------------|---------|---------|----------|
| Corn (8%CP)                   | NR1     | NR2     | NR1      | NR2     | NR1   | NR2   |
| Soybean meal (46%CP)          | 31.04   | 28.76   | 27.08    | 23.57   | 23.03 | 23.97 |
| Bone and meat meal (45%CP)    | 4.00    | 4.00    | 4.00     | 4.00    | 4.00  | 4.00  |
| Soybean oil                   | 1.42    | 1.00    | 3.21     | 1.51    | 3.15  | 3.37  |
| Dicalcium phosphate           | 0.85    | 0.86    | 0.65     | 0.67    | 0.50  | 0.49  |
| Limestone                     | 0.61    | 0.62    | 0.55     | 0.55    | 0.50  | 0.50  |
| Salt                          | 0.37    | 0.37    | 0.34     | 0.34    | 0.31  | 0.31  |
| L-Lysine HCl (78%)³           | 0.35    | 0.12    | 0.26     | 0.20    | 0.32  | 0.13  |
| DL-Methionine (99%)³          | 0.33    | 0.22    | 0.26     | 0.23    | 0.25  | 0.21  |
| L-Threonine (98%)³            | 0.14    | 0.01    | 0.08     | 0.04    | 0.10  | 0.00  |
| Sodium bicarbonate            | 0.10    | 0.10    | 0.10     | 0.10    | 0.10  | 0.10  |
| Kaolin³                       | 0.10    | 0.10    | 0.08     | 0.08    | 0.10  | 0.10  |
| Mineral supplement⁴           | 0.10    | 0.10    | 0.10     | 0.10    | 0.10  | 0.10  |
| Vitamin supplement⁵           | 0.10    | 0.10    | 0.10     | 0.10    | 0.10  | 0.10  |
| Salinomycin                   | 0.05    | 0.05    | 0.05     | 0.05    | 0.05  | 0.05  |
| Zinc bacitracin               | 0.03    | 0.03    | 0.03     | 0.03    | 0.03  | 0.03  |
| Protease                      | 0.02    | 0.02    | 0.02     | 0.02    | 0.02  | 0.02  |
| Total                         | 100.00  | 100.00  | 100.00   | 100.00  | 100.00| 100.00|

| Calculated composition of nutrients | Starter | Groover | Finisher |
|-------------------------------------|---------|---------|----------|
| Crude Protein (%)                   | 21.47   | 19.97   | 19.50    | 18.38   | 18.21 | 17.33 |
| Met. Energy kcal/kg                 | 3050    | 3050    | 3150    | 3150    | 3200  | 3200  |
| Dig. Lysine (%)                     | 1.26    | 1.03    | 1.10    | 0.97    | 1.05  | 0.92  |
| Dig. Methionine (%)                 | 0.62    | 0.50    | 0.53    | 0.49    | 0.50  | 0.47  |
| Dig. Methionine+cystine (%)         | 0.90    | 0.78    | 0.80    | 0.74    | 0.76  | 0.72  |
| Dig. Threonine (%)                  | 0.82    | 0.67    | 0.73    | 0.63    | 0.68  | 0.60  |
| Calcium (%)                         | 0.92    | 0.92    | 0.83    | 0.83    | 0.77  | 0.77  |
| Available P (%)                     | 0.46    | 0.46    | 0.41    | 0.41    | 0.38  | 0.38  |
| Sodium (%)                          | 0.22    | 0.22    | 0.21    | 0.21    | 0.20  | 0.20  |
| Chlorine (%)                        | 0.20    | 0.20    | 0.20    | 0.20    | 0.20  | 0.20  |

¹ NR1: Rostagno et al., (2005).
² NR2: Cobb-Vanterss (2008).
³ Ingredients and nutrients that had a variation in the experimental diets.
⁴ Mineral Premix: Zinc (110.000 mg), Selenium (360 mg), Iodine (1.400 mg), Copper (20.000 mg), Manganese (156.000 mg), Iron (96.000 mg).
⁵ Vitamin Premix: Vitamin K3 (6.000 mg), Vitamin B12 (40.000 µg), Niacin (75.000 mg), Vitamin B1 (5.000 mg), Folic acid (3.000 mg), Pantothenic acid (30.000 mg), Biotin (150 mg), BHT (100 mg), Calcium (82 g), Vitamin B6 (8.000 mg), Vitamin A (25.000.000 UI), Vitamin D3 (6.000.000 UI), Vitamin and (45.000 UI), Vitamin B2 (12.000 mg).
fermentation of (15000 PROT). This protease is manufactured from the Bacillus licheniformis, to the manufacturer’s recommendation – 200 ppm protease units/g of enzyme, and was added according to the recommendation proposed for each stage. According to the recommendation of Rostagno et al. (2005), the amino acid profile was calculated using the ideal protein concept, establisher lysine as the reference amino acid. The amino acids recommendations of the Cobb-Vantress Guidelines (2008) were followed except for threonine that was calculated according to the ideal protein concept.

Ingredients that underwent alterations in the reductions of amino acids amounts (AR) were: L-Lysine HCl; DL-Methionine; L-Threonine; inert material and protease). Their respective ratios and nutritional compositions are shown in Table 2 for the broilers rearing periods: starter (1 to 21 days), grower (22 to 35 days), and finisher (36 to 42 days). Amounts of the other ingredients were maintained.

The performance variables that were analyzed: feed intake (g), weight gain (g), and feed conversion ratio (g/g). The broilers were weighed on days 1, 21 and 42. To evaluate the slaughter weight, yield of carcass, breast, thigh + drumstick, wing and abdominal fat, two birds were selected at 42 days of age per experimental group, based on the group's average body weight (± 5%). After an 8-hour fasting period, the broilers were desensitized by electronarcosis, and then slaughtered in a room with blue artificial light. They were eviscerated to evaluate and calculate the yield of carcass, breast, thigh + drumstick, wing and abdominal fat. The carcass yield was quantified adding the head, neck and feet weight. The absolute weight of pancreas was measured to determine if there were alterations resulting from the protease supplementation.

The energy and mineral levels were adjusted so all the diets would have the same amount of energy and nutrients for each period of the broiler’s life. Only the amino acid levels were different based on the nutritional recommendation proposed for each stage. The first amino acid reduction (AR1), lysine, methionine and threonine were previously based on the true amino acid digestibility of the ingredients (corn - 8% CP; soybean meal – 46% CP; and meat and bone meal – 45% CP) supplemented with protease according to study of Pinto (2011), from 14 to 21 d and 36 to 42 d. In the second (AR2) and third (AR3) amino acid reduction, the true amino acid digestibility of the feed ingredients were overestimate in 20 and 40%, respectively, in the diets supplemented with protease from 14 to 21 d and 36 to 42 d (Table 2). The amino acid reduction diets were formulated according to Rostagno et al. (2005) (NR1) and Cobb-Vantress Guidelines (2008) (NR2) recommendations.
RESULTS AND DISCUSSION

There was no significant interaction ($p<0.05$) between the reductions in digestible amino acids (methionine, lysine and threonine) amounts and the two nutritional recommendations for the performance variables of broilers from 1 to 21 and from 1 to 42 days of age (Table 3). However, the recommendations of Rostagno et al. (2005) increased weight gain and decreased feed conversion ratio ($p<0.05$) when compared to the Cobb-Vantress Guidelines (2008) in all evaluated periods.

Toledo et al. (2007) evaluated two nutritional densities (standard and low), by adding enzymes (amylase, xylanase and protease), in the starter period and found no significant interaction between the nutritional densities and the enzyme complex on performance variables and bioeconomic index. According to Oxenboll et al. (2011), including protease in broiler diets with different protein levels does not

Table 2 – Ingredients that underwent alterations in the experimental diets for the starter (1 to 21 days), grower (22 to 35 days) and finisher (36 to 42 days) periods of broiler rearing according to two nutritional recommendations (NR) and three reductions in the amount of amino acids (AR).

| Ingredients (g.kg$^{-1}$) | AR1 | AR2 | AR3 | AR1 | AR2 | AR3 |
|---------------------------|-----|-----|-----|-----|-----|-----|
| L-Lysine HCl$^1$          | 0.3240 | 0.3190 | 0.3135 | 0.1035 | 0.0990 | 0.0953 |
| DL-Methionine$^1$         | 0.2849 | 0.2760 | 0.2679 | 0.1851 | 0.1780 | 0.1706 |
| L-Threonine$^1$           | 0.0518 | 0.0340 | 0.0167 | 0.0000 | 0.0000 | 0.0000 |
| Inert material$^1$        | 0.2366 | 0.2680 | 0.2992 | 0.1436 | 0.1550 | 0.1663 |
| Protease$^1$              | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 0.0200 | 0.0200 |
| Calculated Composition of Nutrients(%) |
| Dig. Lysine$^1$           | 1.2410 | 1.2370 | 1.2330 | 1.0190 | 1.0160 | 1.0110 |
| Dig. Methionine$^1$       | 0.6010 | 0.5980 | 0.5950 | 0.4920 | 0.4900 | 0.4880 |
| Dig. Meth.+Cyst.$^1$      | 0.8540 | 0.8460 | 0.8370 | 0.7410 | 0.7340 | 0.7270 |
| Dig. Threonine$^1$        | 0.7410 | 0.7250 | 0.7090 | 0.6660 | 0.6660 | 0.6660 |

1Ingredients and nutrients that had a variation in the experimental diets.

AR1- Amino acids reduction 1 (with protease and amino acids reduction, considering real digestibility); AR2- Amino acids reduction 2 (with protease and amino acids reduction, considering 20% higher digestibility); AR3- Amino acids reduction 3 (with protease and amino acids reduction, considering 40% higher digestibility).
affect feed intake and feed conversion ratio at 42 days of age. Freitas et al. (2011) evaluated different nutritional plans with variable metabolizable energy and crude protein in diets with or without protease supplementation. The authors found no effect on the body weight of broilers at 42 days, and a quadratic effect on feed conversion ratio when protease was included in the formulation. Broilers fed diets with the highest protein level had better performance. Angel et al. (2011) reported similar results in broilers up to 21 days of age. According to these authors, protease was more active with a higher amount of protein substrate and the same amount of metabolizable energy.

Based on data of the present study, it was found that adding protease in diets for broilers lead to the possibility of considering the digestibility of amino acids (lysine, methionine, and threonine) present in feed ingredients up to 40% higher than the real digestibility, independently of crude protein level and synthetic amino acids used in different nutritional plans. According to Freitas et al. (2011), the effect of supplementing protease is more pronounced in broiler diets with higher amounts of crude protein. This statement is in agreement with the results obtained in this study, where the nutritional recommendations by Rostagno et al. (2005), supplying a higher amount of crude protein than recommended by the Cobb-Vantress Guidelines (2008), provided better results for the same performance variables evaluated.

According to Silva et al. (2001) the lower ratio of metabolizable energy and crude protein (ME:CP) affect performance increasing weight gain. It directly influences the pool of amino acids for protein deposition and uric acid synthesis as well as in the supply of carbohydrate and fat to meet the energetic requirements of the broiler chickens. According to the NRC (1994) the ME:CP for broilers should be 160 for the highest performance. In the present study, it was observed that the average ME:CP was 158 and 168 by recommendation of Rostagno et al. (2005) and Cobb-Vantress (2008), respectively. This fact also contributed to the improvements of broilers

Table 3 – Performance of broilers fed diets formulated according to two nutritional recommendations with different reductions of digestible amino acid amounts with protease supplementation from 1 to 21 and 1 to 42 days of age.

| Amino acid reduction (AR) | 1 to 21 days | 1 to 42 days |
|---------------------------|--------------|-------------|
|                           | WG (g)       | FI (g)      | FCR (g/g) | WG (g)       | FI (g)      | FCR (g/g) |
| AR1                       | 838          | 1237        | 1.48      | 2788         | 4890        | 1.75      |
| AR2                       | 847          | 1245        | 1.47      | 2767         | 4893        | 1.77      |
| AR3                       | 841          | 1237        | 1.47      | 2774         | 4995        | 1.78      |
| Nutritional recommendation (NR) |          |             |           |             |             |           |
| Rostagno et al. (2005)    | 855 a        | 1243        | 1.45 a    | 2831 a       | 4901        | 1.73 a    |
| Cobb-Vantress (2008)      | 828 b        | 1236        | 1.49 b    | 2728 b       | 4902        | 1.80 b    |
| p value                   |              |             |           |              |             |           |
| AR                        | 0.6258       | 0.7343      | 0.8065    | 0.6193       | 0.3515      | 0.1041    |
| NR                        | 0.0015       | 0.5043      | <0.0001   | <0.0001      | 0.4579      | <0.0001   |
| AR x NR                   | 0.7303       | 0.5295      | 0.8195    | 0.2085       | 0.9383      | 0.2426    |
| CV (%)                    | 2.64         | 2.40        | 1.69      | 1.96         | 2.07        | 1.85      |

WG = weight gain; FI = feed intake; FCR = feed conversion ratio; CV = coefficient of variation (%); P value = probability; AR x NR = interaction between the reduction in digestible amino acid amounts and nutritional recommendations. Means followed by different letters in the columns are different according to Tukey's test (p<0.05). AR1- Digestible amino acids reduction 1 (with protease and amino acid reduction considering the ingredients real digestibility (Pinto, 2011); AR2- Digestible amino acids reduction 2 (with protease and amino acid reduction considering ingredients digestibility 20% higher than true); AR3- Digestible amino acids reduction 3 (with protease and amino acid reduction considering ingredients digestibility 40% higher than true).
performance fed diets formulated according to Rostagno et al. (2005).

It is known that broilers require essential amino acids and not crude protein and nitrogen amounts that are sufficient for the synthesis of non-essential amino acids (Vasconcellos et al., 2010). It is also known that protein absorption from the intestinal lumen takes place as di- and tripeptides. This could be the reason for a better broiler performance with a high-protein diet. The enzyme-substrate specificity and non-standardization of protein feed ingredient, mainly from animal origin, may be an explanation for the absence of the effect on broiler performance in the diets supplemented with exogenous serine-protease (Vieira et al., 2004; 2016), as in the present study.

Besides that, one of the main factors is the level of digestible lysine in the diet as all other limiting amino acids are calculated as a function of lysine. Thus, when there is an unbalance of a certain digestible amino acid, namely lysine, there are deamination and transamination reactions with donation and/or degradation of its respective amino groups, moving the amino acid function to other functions such as excretion of its metabolism products (Dalólio et al. 2016). Rodrigues et al. (2008) claim that the digestible lysine:crude protein ratio in the diet is very important and should be maintained around 5.90% until 42 days. In the present study this ratio was 5.77% and 5.31% in the Rostagno et al. (2005) and Cobb-Vantress Guidelines (2008), respectively. Goulart et al. (2011) stressed that the methionine+cystine:digestible lysine ratio in the grower phase should be around 72%, as recommended by Rostagno et al. (2005), while in the Cobb-Vantress Guidelines (2008), this ratio was 76.30%.

Thus, the methionine+cystine concentration imbalance in the diet could be a determinant factor in limiting the growth of broilers fed diets formulated according to the recommendations for the Cobb-500 strain.

As to the carcass characteristics and absolute weight of the pancreas at 42 days (Table 4), there was no isolated effect or a significant interaction ($p>0.05$) between the reduction of synthetic amino acid (lysine, methionine and threonine) and the two nutritional recommendations, Rostagno et al. (2005) and Cobb-Vantress (2008). There was effect ($p<0.05$) of amino acid reduction and protease supplementation only at slaughter weight, being that when overestimating the digestibility of the ingredients (corn, soybean meal and meat and bone meal) of the diet up to 40% (AR3) the slaughter weight was lower in relation to the digestibility of the ingredients (AR1). Dosković et al. (2016) did not observe effect on slaughter weight of 49-day-old broiler chickens fed diets supplemented with protease and different levels of crude protein. According to Makhdum et al. (2013), slaughtering weight tends to be higher when supplementing enzymes in diets based on high-fiber feed ingredients. However, this fact did not occur in the present study, where the diets were based on corn, soybean meal and meat-and-bone meal. Kamel et al. (2015) reported that the highest level of the major crystalline amino acids (methionine, lysine and threonine) provided in the diets associated with protease supplementation for broilers were the factors that most affected the carcass characteristics, regardless of metabolizable energy level and crude protein. This may led to an increase in the weight of the broiler's viscera, without altering the carcass yield and noble cuts.

Table 4 – Carcass characteristics of broilers at 42 days of age fed diets formulated according to two nutritional recommendations with different reductions of digestible amino acid amounts with protease supplementation.

| Amino acid reduction (AR) | SW (g) | CY* (%) | BY (%) | TDY (%) | AF (%) | P (g) |
|--------------------------|--------|---------|--------|---------|--------|-------|
| AR1                      | 2885 a | 82.87   | 33.85  | 25.62   | 1.73   | 5.02  |
| AR2                      | 2791 ab| 83.30   | 32.66  | 26.22   | 1.66   | 5.00  |
| AR3                      | 2707 b | 82.41   | 32.64  | 26.06   | 1.78   | 5.07  |
| Nutritional Recommendation (NR) |       |         |        |         |        |       |
| Rostagno et al. (2005)   | 2791   | 82.58   | 33.28  | 25.70   | 1.69   | 5.00  |
| Cobb-Vantress (2008)     | 2797   | 83.13   | 32.41  | 26.16   | 1.77   | 5.10  |

* SW = slaughter weight; CY* = carcass yield with head, neck and feet; BY = breast yield; TDY = thigh and drumstick yield; AF = abdominal fat; P = pancreas; CV = coefficient of variation (%); p value = probability; AR x NR = interaction between the reduction in digestible amino acid amounts and nutritional recommendations. Means followed by different letters in the columns are different according to Tukey’s test ($p<0.05$). AR1- Digestible amino acids reduction 1 (with protease and amino acid reduction considering ingredients digestibility 40% higher than true); AR2- Digestible amino acids reduction 2 (with protease and amino acid reduction considering ingredients digestibility 20% higher than true); AR3- Digestible amino acids reduction 3 (with protease and amino acid reduction considering ingredients digestibility 40% higher than true).
Carcass yield results found in this trial are in agreement with findings of several other authors, as they also did not find any influence of reducing crude protein levels of the diet on these parameters, when followed by amino acid or exogenous enzyme supplementation (Kamram et al., 2008; Rodrigues et al., 2008; Vasconcellos et al., 2010). Yuan et al. (2017) reported an increase in the gene expression of pancreas enzymes when a protease supplement was added to the diet for broilers. Lima et al. (2012) identified an increase in the absolute weight of pancreas in layers when they received an enzyme complex (cellulase, pentosanase, pectinase, amylase, protease, β-glucanase, phytase) in the diet. In the present trial no effect of amino acid reduction and protease supplementation of the diet were found on the weight of pancreas of broilers at 42 days.

Rodrigues et al. (2008) evaluated different digestible lysine:crude protein ratios and did not find differences in broilers carcass yield. Vasconcellos et al. (2010) studied different levels of crude protein in broiler diets and found no effect on carcass yield. Levels of crude protein, however, had a quadratic effect on breast yield, and the maximum effect was estimated to be 18.28% between days 22 and 42. In the present study, the average of crude protein content in the diets for broilers from days 22 and 42 was 18.85% and 17.86%, respectively, for the nutritional recommendations of Rostagno et al. (2005) and the Cobb-Vantress Guidelines (2008). However, there were no effects on carcass and breast yield, which is the main meat cut with higher protein concentration in the carcass.

CONCLUSIONS

It is possible to reduce the inclusion of crystalline amino acids (lysine, methionine and threonine) in the diets supplemented with protease, considering that their digestibility is up to 40% higher than the real digestibility when corn, soybean meal and meat and bone meal are used as ingredients. There are differences in relation to the nutritional recommendations for broilers, and the levels recommended by Rostagno et al. (2005) providing better performance, with no effect on carcass yield and broiler cuts when compared to the Cobb-Vantress Guidelines (2008).

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