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Protein and Amino Acid Apparent Ileal Digestibility in Broilers Fed Mucuna (*Mucuna deeringiana [Bort* Merr.]) Foliage Meal in the Diet

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Abstract: Thirty-six broiler chickens (COBB comercial line) four-week old were used to assess the apparent ileal digestibility of protein and amino acids with the dietary inclusion of mucuna (*Mucuna deeringiana*) foliage meal. The animals were distributed according to a completely randomized design with 4 treatments and 3 replicates: control (maize-soybean) and 3 dietary mucuna foliage meal levels of inclusion: 50, 100 and 150 g kg^{-1}. The apparent ileal digestibility of Crude Protein (CP) and 14 amino acids was determined.The protein digestibility was lower ($p<0.05$) in both 50 and 150 g kg^{-1} of mucuna inclusion than in the control diet. The coefficients of apparent ileal digestibility of amino acids in birds fed 150 g kg^{-1} mucuna foliage meal were lower ($p<0.05$) than in those fed the control diet. It is outstanding that there were low apparent ileal digestibilities of glycine, tyrosine and proline with 50 g kg^{-1} of mucuna inclusion in diets. No differences were observed between the control and 100g kg^{-1} mucuna inclusion diets for amino acid digestibilities except for lysine, aspartic acid and valine. It was discussed the role of the dietary fibre content and the presence of anti-nutrient factors as responsible of findings. It is concluded that dietary inclusion up to 100 g kg^{-1} mucuna foliage meal did not affect apparent ileal digestibility of most amino acids studied in the current research.

Key words: Protien and amino acid, digestibility, broiler, foliage meal, CP, Cobb broiler

INTRODUCTION

The growing need for an efficient and low-cost non-ruminant production in the tropics leads to the selection of alternative raw materials, with acceptable bio-availability and do not compete with human feeding (Savón, 2005). There are several ways and alternatives for solving this issue, one of them with higher possibility is the production and use of foliage resources as protein and energy sources (Dung *et al.*, 2002). The utilization of legumes for animal feeding constitutes one of the most accurate alternatives. The foliage of *Stizolobium* sp. (mucuna) is an outstanding resource due to its crude protein content (CP: 18.79%) (Martínez *et al.*, 2007) and also to the mineral (Díaz *et al.*, 2002) levels, which makes it attractive for its inclusion in poultry diets. However, as a non-conventional feed, it also contents an important amount of neutral detergent fibre (NDF: 46.85%) and Anti-Nutrient Factors (ANFs) (Scull, 2004), which diminish the apparent digestibility of the nitrogenous compounds (Savón, 2005). There is little information related to the effect of the foliage of this legume on digestive features in poultry, because most of the results are focused on the productive performance. Therefore, this study was aimed to evaluate the apparent ileal digestibility of protein and amino acids in broilers fed mucuna (*Stizolobium deeringiana*) foliage meal in the diet.

MATERIALS AND METHODS

Animals and diets: Thirty-six male Cobb broilers 4 week old (1.3±0.1 kg body weight) were used. The birds were housed randomly in wire cages (40×40×80 cm) having a feeder and a drinker. The water and food were offered ad libitum. The four experimental treatments (nine birds each) consisted of a control diet (corn-soybean) and three dietary levels (50, 100 or 150 g kg^{-1}) of *Stizolobium deeringiana* foliage meal (mucuna) cv. Pinto (Table 1). The energy: protein ratio was kept constant in all diets. Titanium dioxide (TiO_2) was used as a dietary marker including 4 g kg^{-1} (Short *et al.,* 1996). Mucuna foliage was harvested between 70 and 80 d of age at 5 cm above the soil level. It was dried in an oven at 60°C for 48 h and then

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ground through a 3 mm mesh sieve by an electric hammer mill (Christy). It contained 938.5 g kg^{-1} dry matter, 187.9 g kg^{-1} crude protein, 223.0 g kg^{-1} crude fibre and 90.5 g kg^{-1} ash.

Experimental procedure: The experiment was performed at the Faculty of Veterinary Medicine and Animal Science of the Autonomous University of Yucatan, Mexico (FMVZ-UADY). The birds were fed for 14 day and then, they were slaughtered by an intravenous injection of sodium pentobarbitone and abdominal cavity was opened to locate the ileum, defined as the portion of the small intestine extended from the Meckel´s diverticulum up to the ileum-cecal joint. The content was extracted manually, using the forefinger and the thumb throughout the ileum and later deposited in Petri dishes (Sebastian *et al.*, 1997). The ileal content from every three chickens was pooled and taken as a repetition. The samples were freeze dried and then chemical analyses were performed.

Chemical analyses: The determination of TiO_2 was performed according to the colorimetric method described by Short *et al.* (1996). The analysis of amino acids was carried out by high performance liquid chromatography (HPLC) (Krishnamurti *et al.*, 1984). For the digestion of the sample, the method of pre-column derivatization with DABS-Cl was used. The hydrolysis was conducted with HCl 6N at 110°C for 20 h (Knecht and Chang, 1986). Norleucine was used as internal standard.

The digestibility coefficient for each amino acid was calculated according to the formula proposed by Kadim and Moughan (1997) and Ravindran *et al.* (1999).

Experimental design and statistical analysis: Analysis of variance was performed on the data according to a completely randomized design with 4 treatments and 3 replicates (3 birds each) by treatment. Differences between means were estimated by Duncan (1955) test. Infostat (2001) version 1.0 was used as statistical software.

Table 1: Composition of experimental diets and chemical analyses (g kg^{-1})

Ingredients	Control	Mucuna 50	Mucuna 100	Mucuna 150
Maize	659.0	613.0	566.9	520.9
Soyabean meal	298.8	283.1	267.3	251.5
Ca_2CO_3	15.7	15.5	15.3	15.0
NaCl	2.5	2.5	2.5	2.5
Vitamin pre-mixture[1]	0.3	0.3	0.3	0.3
Calcium phosphate	10.2	10.8	11.5	12.2
Sunflower oil	13.1	24.4	35.7	47.1
Mucuna	-	50	100	150
Mineral pre-mixture[2]	0.5	0.5	0.5	0.5
Chemical analysis				
Dry matter	903.2	905.7	908.2	912.5
Crude protein	194.4	193.7	185.0	185.1
Crude fibre	23.0	33.3	40.5	56.2

[1]:Mineral Premix g kg^{-1}: Mn, 65 mg; I, 1 mg; Fe, 55 mg, Cu, 6 mg; Zn, 55 mg; Se, 0.3 mg. 700 g kg^{-1} of Choline chloride; [2]:Vitamin Premix g kg^{-1}: vitamin A, 8000 UI; vitamin D, 2500 UI; vitamin E, 8 UI; vitamin K, 2 mg; vitamin B_{12}, 0.002 mg; riboflavin, 5.5 mg; calcium pantothenate 13 mg; niacine, 36 mg; Choline chloride, 500 mg; folic acid, 0.5 mg; thiamine, 1 mg; pyridoxine, 2.2 mg; biotin, 0.05 mg

RESULTS

The crude protein and amino acid concentrations of diets are shown in Table 2. The protein digestibility was lower ($p<0.05$) with 50 and 150 g kg^{-1} of mucuna inclusion than in the control diet. The coefficients of apparent ileal digestibility of amino acids in birds fed 150 g kg^{-1} of mucuna foliage meal were lower ($p<0.05$) than in those fed the control diet. It is noteworthy that there were low apparent ileal digestibilities of glycine, tyrosine and proline with 50 g kg^{-1} of mucuna inclusion in diets. However, no differences were observed between the control and 100 g kg^{-1} mucuna inclusion diets for amino acid digestibilities except for lysine, aspartic acid and valine (Table 3).

Table 2: Crude protein and amino acid contents of diets and mucuna foliage meal (g kg^{-1}) on dry basis

	Diets				
Parameter	Control	50	100	150	Mucuna foliage meal
Crude protein	211.8	190.0	196.0	189.3	187.9
Alanine	12.3	14.0	12.0	12.3	10.7
Arginine	11.9	12.0	11.2	11.2	6.9
Aspartic acid	17.4	13.7	16.8	17.8	13.1
Glutamic acid	39.6	29.7	36.0	37.1	19.9
Glycine	12.4	12.0	11.6	12.4	11.9
Histidine	9.3	11.1	8.8	9.2	8.4
Isoleucine	14.0	13.9	12.9	13.1	12.0
Leucine	23.3	24.1	21.0	20.9	17.0
Lysine	10.9	10.7	10.9	11.2	22.1
Phenylalanine	14.3	14.4	12.9	12.9	12.0
Proline	26.6	16.4	20.9	21.3	18.4
Serine	11.9	13.1	11.2	11.2	7.7
Tyrosine	14.8	9.0	10.1	10.6	11.3
Valine	15.8	16.1	14.2	15.0	14.2

Table 3: Coefficients of apparent ileal digestibility of the protein and the amino acids in broilers fed different dietary levels of mucuna foliage meal

	Treatments[1]					
Parameter	Control	50	100	150	SE	Probability
CP	0.75[a]	0.58[b]	0.66[ab]	0.57[b]	0.03	0.0120
Alanine	0.79[a]	0.71[a]	0.70[a]	0.60[b]	0.02	0.0071
Arginine	0.85[a]	0.79[ab]	0.79[ab]	0.72[b]	0.02	0.0325
Aspartic acid	0.83[a]	0.70[c]	0.76[b]	0.68[c]	0.01	0.0013
Glutamic acid	0.85	0.82	0.79	0.70	0.04	0.2028
Glycine	0.72[a]	0.57[b]	0.61[ab]	0.50[b]	0.03	0.0141
Histidine	0.71[a]	0.65[a]	0.61[a]	0.47[b]	0.03	0.0123
Isoleucine	0.76[a]	0.66[ab]	0.67[ab]	0.59[b]	0.03	0.0257
Leucine	0.77[a]	0.69[ab]	0.69[ab]	0.60[b]	0.02	0.0149
Lysine	0.73[a]	0.79[a]	0.61[b]	0.60[b]	0.03	0.0063
Phenylalanine	0.73	0.67	0.68	0.58	0.03	0.0669
Proline	0.68	0.33	0.53	0.38	0.08	0.0626
Serine	0.81[a]	0.76[a]	0.71[a]	0.58[b]	0.03	0.0114
Tyrosine	0.68[a]	0.31[c]	0.55[ab]	0.41[bc]	0.06	0.0118
Valine	0.75[a]	0.64[b]	0.65[b]	0.56[b]	0.02	0.0159

[a,b,c] Values with different superscripts within the same row differ significantly at $p<0.05$; [1] The values are means of 3 replicates (3 birds each)

DISCUSSION

The coefficients of digestibility with the inclusion of 150 g kg^{-1} of mucuna foliage meal in the ration were low. Similar results were found in broilers by Monforte *et al.* (2006) and Sarmiento *et al.* (2003) who observed low digestibility by birds in fibrous foodstuffs such as wheatfeed and chaya (*C. aconitifolius*) leaf meal, respectively. Those findings could be attributed to an increased rate of passage due to the presence of insoluble fibre in the mucuna foliage (Martínez *et al.*, 2007) and consequently decreasing the protein digestion and increasing the secretion of endogenous amino acids (Yin *et al.*, 2000; Arija *et al.*, 2006).

Savón (2005) stated that the effect of the insoluble dietary fibre on the intestinal motility depends on its amount in the diet. Seemingly, the dietary fibre threshold allowed for this species is exceeded quantitatively with 150 g kg^{-1} of mucuna foliage meal inclusion.

Another factor that may contribute to the decrease of digestibility of amino acids was the presence of ANFs in the ingredient. There are tannins and phenolic substances of high molecular weight (Scull, 2004) in the mucuna foliage, which can form compounds with the proteins and the structural carbohydrates (Martínez-Valverde *et al.*, 2000). These compounds impair the metabolism of nutrients and the interactions enzymes-tannins, inhibit the activity of the digestive enzymes and influence the absorption by means of their astringent action, leading to a reduction in the digestibility (Ravindran *et al.*, 2005; Savón, 2005).

The relatively low apparent ileal digestibilities of glycine, tyrosine and proline may, in part, result from their relatively high concentration in endogenous secretions, as reported by Li *et al.* (1994) and Villamide and San Juan (1998). Perhaps the presence of the mucuna fibre promotes this performance.

The reduction of digestibility coefficients of lysine, aspartic acid and valine, in both 100 and 150 g kg^{-1} dietary mucuna foliage meal inclusions could be explained by diferent hypotheses. Lysine could probably combine with ANFs forming indigestible compounds, according to Ravindran *et al.* (2005) in a study with cottonseed meal in broilers.

On the other hand, aspartic acid is an important amino acid in the microbial cells and an increase in the microbial activity in the intestine, as a consequence of the dietary fibre, probably results in reducing its digestibility (Sarmiento *et al.*, 2003). Valine has a long aliphatic chain of three carbons that is hydrophobic, consequently forming conglomerates (Stryer, 1995), which may represent a physical barrier to its absorption in the small intestine, leading to a reduced digestibility.

The results permit to conclude that dietary inclusion up to 100 g kg^{-1} mucuna foliage meal did not affect apparent ileal digestibility of the protein and most of the amino acids studied in the current work.

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