

## Meal of *Mucuna deeringiana* forage on some physiological microbial groups and fermentative indicators of the cecum of broiler chickens

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An experiment was performed according to complete random design to determine the effect of including the meal of the *Mucuna deeringiana* forage on some physiological microbial groups and on the fermentative indicators of the cecum of broiler chickens. Sixteen male chickens (hybrid HE<sub>21</sub>) were used at a rate of four chickens per treatment. The treatments were: the control, 5, 10, and 15 % of meal of mucuna forage to substitute the corn meal. At 42 d, the microbial groups (total bacteria, proteolytic bacteria, cellulolytic bacteria, and fungi) and the fermentative indicators (SCFAt, ammonia, and pH) were determined. The total bacteria were reduced with 10 % of mucuna, and with 5 %, counts superior to 10<sup>10</sup> ufc mL<sup>-1</sup> were attained. The counts of cellulolytic fungi were not affected, as compared with the control, but they differed between 5 and 10 %, being inferior in the latter (P < 0.05), whereas the cellulolytic bacteria did not show differences between treatments. The proteolytic bacteria were superior (P < 0.05) with 10 %, but there were not differences in respect to the control (2.91 vs 2.33 x 10<sup>6</sup> cfu mL<sup>-1</sup>). The pH did not vary between treatments, the concentration of SCFAt was increased with the inclusion of mucuna, and a peak (82.32 meq L<sup>-1</sup>) was reached with 10 %, whereas the NH<sub>3</sub>, with 10 and 15 % of mucuna, showed lower concentrations, as compared with the rest of the treatments (P < 0.05). It was concluded that the inclusion of the meal of *M. deeringiana* forage increases the production of total SCFA; and, with the highest levels, it diminishes the ammonia in the ceca. In the microbial concentrations, there was not a defined performance being in correspondence with the fermentative indicators.

Key words: *mucuna*, *cecum microorganisms*, *fermentation products*.

In the tropics, there are forage resources that, in middle or small productions, can be promising sources for the feeding of monogastric species (Savón *et al.* 2004a). Among these resources, the forages of legumes and of other trees are outstanding, representing protein and fiber sources. At present, their inclusion in the diet is limited, due to the low information availability about their nutritive value and digestive utilization.

The plants of the genus *Mucuna* are considered good-quality roughage. They are outstanding for their content of crude protein (CP: 20.79 %) (Martínez *et al.* 2007), ashes, and minerals (Díaz *et al.* 2002), which makes them attractive for their inclusion in poultry diets. Nevertheless, as non-conventional feed, they have the inconvenient of the high fiber content (NDF: 46.85 %) and the presence of anti-nutritional factors (ANFs) (Scull 2004).

Diet fiber is the most abundant colon nutrient fibra, and it is totally fermented by microorganisms (Bomba *et al.* 2006 and Roberfroid 2008). The cellulolytic bacteria, that show adaptation response to the fiber in the diet (Rodríguez *et al.* 2000), constitute the physiological group that takes part mainly in its digestibility, although it has been proved the presence of cellulolytic fungi in the cecum of broiler chickens that, due to their colonization form, contribute the most to the degradation of these fibrous materials (Rodríguez *et al.* 1996). However, the magnitude of the response can be affected by the physico-chemical composition of the fiber present in

the diet (Savón *et al.* 2007a).

According to Martínez (2010), the forage of *Mucuna deeringiana* has a chemical and physical composition different to that of other species, thus, its utilization as energy source can provoke modifications in the intestinal ecology of the animals consuming it. From the chemical point of view, it has higher content of DM, CF, EE, and lower content of cell wall and lignin. In regards to the physical composition, it has higher solubility, buffering capacity, and lower capacity of water absorption. The objective of this study was determining the effect of the inclusion of forage meal of *M. deeringiana* on some microbial physiological groups and cecum fermentative indicators of broiler chickens.

### Materials and Methods

*Elaboration of forage meals.* The mucuna forage was produced on red ferrallitic soil, at the experimental station of pastures in the Institute of Animal Science during the period of 2004- 2007. The complete plant was harvested at 5 cm above the soil level at the flowering stage, between 70 and 80 d of age. It was sundried on a plate for two or three days, until reducing the humidity between 20 and 25 %. It was ground in hammer mill, with 3 mm sieve.

*Experimental procedure.* Sixteen male broiler chickens were used at 18 d of age (hybrid HE<sub>21</sub>), housed in individual cages in the metabolism pen of the Institute of Animal Science. The animals were subject to the experimental diets during the growing-fattening stage.

The treatments consisted of four diets formulated according to the requirements of the NRC (1994) for this poultry category. The cornmeal was substituted, partly, by the meal of mucuna forage (0, 5, 10, and 15 %). The diet was given daily *ad libitum*. During this period, the animals were given water treated with sodium hypochlorite at 0.1%, *ad libitum*. The animals were vaccinated against Gumboro, at 5 and 10 d of age (first and second doses, respectively) and against Newcastle at 12 d (tables 1 and 2)

The animals were slaughtered at 42 d by bleeding through the jugular vein (Sánchez 1990). The cecum was removed and its content was collected under CO<sub>2</sub> atmosphere. Serial dilutions were made up to 10<sup>-10</sup> to determine the different microbial groups.

*Determination of the microbial groups.* The method of the roll tubes was used (Hungate 1966). The media were the following:

- Total bacteria: The medium 10 of Caldwell and Bryant (1966) was used, as modified by Elías (1971). It was cultured on 10<sup>-8</sup> to 10<sup>-10</sup> dilutions.

- Proteolytic bacteria: The same medium was taken as for the total, being added 0.5 % of carboxymethylcellulose and 10 % of skim milk. It was cultured in the dilutions 10<sup>-5</sup> -10<sup>-7</sup>.

- Cellulolytic bacteria: They were grown on medium similar to that of the total, substituting the sources of carbohydrates by powder cellulose, 1% and 0.03 % of celobiose. They were cultured in the dilutions 10<sup>-4</sup> -10<sup>-6</sup>.

- Cellulolytic fungi: A medium was used being similar to that of the cellulolytic bacteria, but adding 0.5 % of carboxymethylcellulose. The bacterial growth

was prevented adding 0.01% of cloramphenicol (Joblin 1981). It was cultured in the dilutions 10<sup>-4</sup> -10<sup>-6</sup>.

*Fermentative indicators.* Before the sample was subject to centrifugation, the pH was measured with digital pH meter, brand WPA, series CD-70, of English manufacture. The concentration of ammonia (NH<sub>3</sub>) was determined according to Conway (1957). The determinations of total short chain fatty acids (total SCFA) were conducted by Pennington (1952). The samples were made appropriate dilutions after centrifugation, at 3 000 r.p.m., one gram of cecal content in 1:10 dilution with distilled water, and later the precipitate was discarded (Ly *et al.* 1980). The sample fermentation was halted adding mercury dichloride at 10 % (0.02 mL).

*Statistical methods.* A complete random design was applied, with four treatments, and four and eight repetitions, for the microbial groups and the fermentative indicators, respectively. The statistical software was INFOSTAT (Balzarini *et al.* 2001), version 5.1 on Windows XP. When necessary, the average values were compared through the test of Duncan (1955).

The counts of viable microorganisms were transformed according to log N to guarantee the normality conditions. For the analysis, the formula (K+N) \*10<sup>x</sup> was used, where,

k - constant representing the logarithm of the dilution being inoculated the microorganism

N- logarithm of the count of colonies, determined as cfu mL<sup>-1</sup>

10 - the base of the logarithms

x- dilution to which the inoculation was performed

When the count was equal to zero, a constant was summed (x + 0.375).

Table 1. Experimental diets corresponding to the growing period (21-35 d of age)

Ingredients	Mucuna in the diet, %			
	0	5	10	15
Corn	62.90	57.90	52.60	47.30
Soybean	29.30	29.00	29.00	29.00
Forage meal of mucuna	-	5.00	10.00	15.00
Soybean oil	4.00	4.30	4.60	4.90
Dicalcium phosphate	1.20	11.20	1.20	1.20
Mineral and vitamin premixture <sup>1</sup>	0.80	0.80	0.80	0.80
Common salt	0.30	0.30	0.30	0.30
Calcium carbonate	1.50	1.50	1.50	1.50
Bromatological analysis (%)				
DM	88.52	86.76	85.14	86.08
CP	20.90	20.40	20.10	20.00
NDF	18.95	20.87	22.83	25.25
ADF	8.99	12.96	15.10	17.85

<sup>1</sup>Vitamin supplement: vitam. A, 10 000 IU; vitam. D3, 2 000 IU; vitam. E, 10 mg; vitam. K, 2 mg; thiamine, 1 mg; riboflavin, 5 mg; pyridoxine, 2 mg; vitam. B12, 15.4 µg; nicotinic acid, 125 mg; calcium panthotenate, 10 mg; folic acid, 0.25 mg; biotin, 0.02 mg.

Mineral supplement: selenium, 0.1 mg; iron, 40 mg; copper, 12 mg; zinc, 120 mg; magnesium, 100 mg; iodine, 2.5 mg; cobalt 0.75 mg.

Table 2. Experimental diets corresponding to the finishing period (35 - 42 d of age)

Ingredients	Mucuna in the diet, %			
	0	5	10	15
Corn	65.70	60.90	56.80	51.80
Soybean	26.70	26.30	25.20	25.00
Forage meal of mucuna	-	5.00	10.00	15.00
Soybean oil	3.80	4.00	4.20	4.40
Dicalcium phosphate	1.00	1.00	1.00	1.00
Mineral-vitamin premixture <sup>1</sup>	1.00	1.00	1.00	1.00
Common salt	0.30	0.30	0.30	0.30
Calcium carbonate	1.50	1.50	1.50	1.50
Bromatological analysis (%)				
DM	90.25	89.3	88.45	88.09
CP	18.71	17.19	17.83	17.45
NDF	19.64	21.23	23.97	26.12
ADF	9.75	12.03	15.38	17.54

<sup>1</sup>Vitamin supplement: vitam. A, 10 000 IU; vitam. D3, 2 000 IU; vitam. E, 10 mg; vitam. K, 2 mg; thiamine, 1 mg; riboflavin, 5 mg; pyridoxine, 2 mg; vitam. B12, 15.4 µg; nicotinic acid, 125 mg; calcium panthotenate, 10 mg; folic acid, 0.25 mg; biotin, 0.02 mg.

Mineral supplement: selenium, 0.1 mg; iron, 40 mg; copper, 12 mg; zinc, 120 mg; magnesium, 100 mg; iodine, 2.5 mg; cobalt 0.75 mg

### Results and Discussion

The microflora of the intestine is a complex community having numerous interactions. Its function is the result from all the components involved (Bomba *et al.* 2006). Table 3 presents the concentration of some microbial groups present in cecum of chickens fed different mucuna ratios.

There was decline in the total bacteria with the 10 % of inclusion of mucuna, and the 5 % was superior to the rest of the treatments. The cellulolytic fungi were not affected, compared with the control, although they differed between the diets with 5 and 10 % of mucuna. They were lower for the latter ( $P < 0.05$ ).

According to Martínez (2010), in the forage meal of *M. deeringiana* there is insoluble fiber. The differences

could be related to the ratios supplied by this type of fiber and to the size of the particles. With 10 and 15 % there is increase in the concentration of insoluble fibrous material in the ration, which could provoke higher speed of intestinal passage. Thus, the time of permanence of the feed decreases for the development of the fermentative processes in the cecum. With 5 %, the animal has more digestible nutrients, supplied by the rest of the ingredients of the diet (corn- soybean). This could favor the entrance of these nutrients into the organ, and could guarantee the growth of the microbiota.

Birds have a mechanism to select particles in the ileocecal valve, which does not permit that particles larger than 1 mm be introduced in the ceca (Denbow 2000). According to Savón *et al.* (2004b), in *Mucuna*

Table 3. Effect of the mucuna inclusion on the microbial growth of the cecum of broiler chickens

Microorganisms <sup>1</sup>	Mucuna in the diet, %				E.S±
	0	5	10	15	
Total bacteria (x 10 <sup>-10</sup> cfu mL <sup>-1</sup> )	5.30 <sup>c</sup> (202.63)	>10 <sup>-10a</sup>	4.46 <sup>b</sup> (90.00)	4.84 <sup>bc</sup> (132.75)	0.17*
Cellulolytic fungi (x 10 <sup>-4</sup> tfu mL <sup>-1</sup> )	2.74 <sup>ab</sup> (17.75)	2.81 <sup>a</sup> (17.75)	2.01 <sup>b</sup> (7.25)	2.74 <sup>ab</sup> (15.75)	0.23*
Cellulolytic bacteria (x 10 <sup>-4</sup> ufc mL <sup>-1</sup> )	2.17 (9.25)	1.76 (6.50)	1.90 (7.50)	1.69 (6.25)	0.34
Proteolytic bacteria (x 10 <sup>-6</sup> cfu mL <sup>-1</sup> )	2.33 <sup>ab</sup> (12.75)	2.00 <sup>b</sup> (8.00)	2.91 <sup>a</sup> (19.00)	1.70 <sup>b</sup> (5.25)	0.28*

<sup>1</sup>Data transformed according to log n

Original means between parentheses

<sup>abc</sup>Different letters in the same row differ significantly at  $P < 0.05$  (Duncan 1955). \* $P < 0.05$

*niveum*, 34.06 % was larger than 1 mm. Maybe, the size of the particles of these legumes could have similar values reducing the possibility of entrance into these organs, straying from the 10 % of forage meal of *Mucuna deeringiana*.

According to Gabriel *et al.* (2007), the complexity of the ecosystem of the large intestine has not permitted determining with accuracy the magnitude of the populations in which they inhabit. However, there are numerous evidences that there is an adaptative response of the cellulolytic microflora in diets of high fiber content, which could represent 10 % of the total population (Varel and Pond 1985, Duke 1997 and Apajalahti and Kettunen 2002).

Nevertheless, the number of colonies of cellulolytic bacteria did not differ between treatments, showing that the inclusion of mucuna did not exert the expected effect on these populations of microorganisms. These results were in agreement with those of Pérez (2006), when including forage meal of *Canavalia ensiformis* in the diet of broiler chickens. Nevertheless, they differed from those of Rodríguez *et al.* (2001), who used the meal of fermented sugarcane (saccharina) in combined cereal diets for pigs. These authors found effect on the cellulolytic bacteria. These differences could be given by the anatomical and physiological characteristics of the species under study and by the type of fiber that differs between the sources.

The forage meal of the legumes has lower hemicellulose-cellulose ratio compared with saccharina (0.47 vs 0.72). In the latter, the fermentative process during its elaboration modifies the ratio of these components. Therefore, the sclerenchymatous tissue is more friable and homogeneous, and provides more chances of cecum fermentation (Marrero 1998).

Elwyn *et al.* (1991) stated that the populations of cellulolytic fungi constitute the more real index for the fermentative processes in the cecum. They are also the responsible for the enzymatic hydrolysis of the fibrous components reaching to this organ, because different to the bacteria, the fungi penetrate the fiber with the growth of their micelle (Bauchop 1988). Thus, they attain a much stronger association.

This agreed with Rodríguez *et al.* (2000), who found in swine diets, that by substituting wheat by saccharina, the specific activity of the cellulase complex was determined by the fungus growth, and not by the cellulolytic bacteria. However, there was not a similar performance with this source, which could be directly related to the physico-chemical composition of the fiber, the presence of anti-nutritional factors and the retention time, among other factors (Dierick *et al.* 1989).

The counts of proteolytic bacteria did not differ from the control ( $P < 0.05$ ), although the treatment with 10 % of forage in the ration did not differ from the rest having mucuna. As to Savón *et al.* (2007b), the protein fractions of the forage meals of the legumes are of

insoluble nature, which makes them less utilizable for the animal. This, together with the endogenous nitrogen from the desquamation of the epithelium or from the increment in the secretions provoked by the fibrous feeds, could favor the growth of this microbial group in the low tract. Thus, as to the previous statement about the intestinal motility, this performance was not reflected in the treatment with 15 %.

The pH did not vary between treatments (table 4). The values were above those of Jiménez *et al.* (2009) in the cecum of poultry (5.8-6.8) fed beet pulp and oat husk. It is likeable that these differences should be due to the physico-chemical characteristics and to the buffering capacity of the fibrous sources, which differ from the forage meal of *Mucuna deeringiana* (Martínez 2010).

The total SCFA concentration was increased with the inclusion of forage meal of mucuna in the ration, compared with the control; and, in the treatment with 10 %, it differed from the rest (table 4).

This response is expected, knowing that the SCFAs are the main product of the fermentation in the ceca (Khedkar and Ouwehand 2006), and it is in correspondence with Dunkley *et al.* (2007), in presence of high contents of fiber in the diet of monogastric animals. Likewise, it confirms the results obtained by Martínez *et al.* (2008 y 2010), when feeding broiler chickens with foliage meal of *Morus alba* and *Lablab purpureus*. These increments in the SCFAs favor the development of the digestive tract of the host animal (González *et al.* 2007).

These results have their explanation in the absorptive processes of these metabolites in the cecum of the animals, and in the fact that they are closely related to the pH. Out of the SCFAs produced, approximately, between 95 and 99 % are absorbed in non-dissociated form by the membrane of the intestinal cell (Varel *et al.* 1984). The gross absorption of the SCFAs is, mainly, of the passive type. This process increases linearly in respect to the amount they are produced (Vispo and Karasov 1997). It is likeable that, with pH values next to neutrality, such as those obtained in this study, most of these metabolites are dissociated, thereby having rise in their concentration in this organ.

In the concentration of  $\text{NH}_3$ , the treatments with 10 and 15 % of mucuna showed lower concentrations compared with the rest ( $P < 0.05$ ) (table 4). This decline may be the result from the use of this metabolite in the synthesis of microbial protein. It is known that in the birds the microflora of the lower intestinal tract has great capacity for the utilization of this substrate (Preest *et al.* 2003). These results agree with those of Rodríguez *et al.* (2000) and Martínez *et al.* (2007) including different fibrous sources in the diet of broiler chickens.

It is concluded that the addition of forage meal of *Mucuna deeringiana* increased the production of total SCFAs, and fall in the ammonia from the cecum, with the highest levels, whereas in the microbial concentrations

Table 4. Fermentative indicators in the cecum of broiler chickens fed forage meal of mucuna

Indicator	Mucuna in the diet, %				E.S ±
	0	5	10	15	
pH	7.40	7.24	7.45	7.43	0.21
Total SCFA (meq L <sup>-1</sup> )	49.91 <sup>a</sup>	72.72 <sup>b</sup>	82.37 <sup>c</sup>	68.06 <sup>b</sup>	2.34*
NH <sub>3</sub> (meq L <sup>-1</sup> )	7.46 <sup>b</sup>	7.49 <sup>b</sup>	6.35 <sup>a</sup>	6.47 <sup>a</sup>	0.30*

<sup>ab</sup>Different letters in the same row differ significantly at P < 0.05 (Duncan 1955)

\*P < 0.05

there was not defined performance corresponding with the fermentative indicators of the organ. It would be interesting to conduct further studies including other sections of the GIT.

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