

## ILEAL AND RECTAL DIGESTIBILITY OF NUTRIENTS IN PIGS FED GRADED LEVELS OF TREATED CANAVALIA (*Canavalia ensiformis*) BEANS

P.L. Domínguez, M. Macías, E. Delgado and J. Ly

Swine Research Institute. PO Box No. 1, Punta Brava. La Habana, Cuba  
email: pldominguez@iip.co.cu

### SUMMARY

*Six intact or three ileorectomized pigs weighing on average 40 kg were assigned to three treatments according to a 3 x 3 Latin square design to determine the effect of pre-soaked, autoclaved and washed *Canavalia ensiformis* beans (0, 5 and 10%, dry basis) on ileal and rectal digestibility of nutrients in pigs fed on sugar cane molasses plus soybean meal based diets. Raw and treated canavalia beans contained 2.01 and 0.25% L-canavanine, and 10.1 and 2.5 mg/g of antitryptic activity, respectively.*

*A non significant ( $P>0.05$ ), slight decrease in ileal N digestibility was found in pigs fed on canavalia beans (76.7, 75.0 and 75.0%) whereas the reverse was true for rectal N digestibility (84.6, 84.8 and 84.9%). The N balance tended ( $P<0.10$ ) to be increased with an increasing level of canavalia beans in the diet (56.1, 59.4 and 62.0% of N intake). Ileal and rectal N digestibility of canavalia beans as estimated by difference were 51.2 and 65.7% respectively.*

*According to the results of the present investigation, the inclusion of ground, soaked, washed and autoclaved canavalia beans up to 10% in diets for pigs only has a slight negative effect on the apparent ileal digestibility of DM, organic matter and energy. In contrast N digestibility is rather low at the ileal level. On the other hand, the energy balance is not negatively affected by the inclusion of the autoclaved canavalia beans up to 10% in the diet. Finally, the balance of N appeared to be favourably influenced by 10% of treated canavalia beans in the diet. This could be caused by changes in the urea cycle activity due to the remaining L-canavanine found in the seeds.*

**Key words:** digestibility, ileum, rectum, pigs, jackbeans, *Canavalia ensiformis*

**Short title:** Digestion of canavalia beans in pigs

## INDICES DE DIGESTIBILIDAD ILEAL Y RECTAL DE NUTRIENTES EN CERDOS ALIMENTADOS CON NIVELES VARIABLES DE GRANOS TRATADOS DE FRIJOL DE CANAVALIA (*Canavalia ensiformis*)

### RESUMEN

*Seis cerdos intactos o tres ileorectostomizados con un peso promedio de 40 kg fueron distribuidos en tres tratamientos de acuerdo con un diseño cuadrado latino 3 x 3 para determinar el efecto de frijoles de *Canavalia ensiformis* molidos groseramente, remojados, tratados en autoclave y después lavados (0, 5 y 10% en base seca) en la digestibilidad ileal y rectal de nutrientes de cerdos alimentados con dietas de mieles de caña de azúcar y harina de soya. Los granos tratados contenían 2.01 y 0.25% de L-canavanina, y 10.1 y 2.5 mg/g de actividad antitriptica.*

*Se halló un decrecimiento no significativo ( $P>0.05$ ), ligero, en la digestibilidad ileal del N a medida que los cerdos ingirieron más frijoles de canavalia (76.7, 75.0 y 75.0% respectivamente) mientras que lo contrario fue cierto para la digestibilidad rectal del N (84.6, 84.8 y 84.9%). En los animales intactos, el balance de N tendió a incrementarse con el aumento del nivel de frijoles de canavalia en la dieta (56.1, 59.4 y 65.3% del consumo de N). La digestibilidad ileal y rectal de los frijoles de cavalia estimada por diferencia fue 51.2 y 65.7% respectivamente.*

*De acuerdo con los resultados de la presente investigación, la inclusión de frijoles de canavalia, remojados, lavados y tratados con autoclave, hasta un 10% en dietas para cerdos, solamente ejerce un ligero efecto negativo en la digestibilidad aparente de la MS, la materia orgánica y la energía. En contraste la digestibilidad ileal del N fue más bien baja. Por otra parte, el balance de energía no se afecta negativamente por la inclusión de frijoles de canavalia remojados y tratados con autoclave hasta un 10% en la dieta. Finalmente, el balance de N parece influenciarse positivamente con 10% de estas semillas en la dieta. Esto pudiera ser causado por cambios en la actividad del ciclo de la urea debido a la L-canavanina remanente hallada en los frijoles de canavalia..*

**Palabras claves:** digestibilidad, ileon, recto, cerdos, frijol de canavalia, *Canavalia ensiformis*

**Título corto:** Digestión de frijol de canavalia en cerdos

## INTRODUCTION

Legume grains are an important source of protein and energy for farm animals. In this connection, *Canavalia ensiformis* (jack bean) could be an alternative as a tropical legume grain for pigs, among other monogastric animals. This assumption is supported by the annual yield, around four tons of seed per ha and ten tons of plants residues per ha (Dixon et al 1983; León et al 1989, 1991).

A particular advantage of canavalia beans, or jackbeans, is its capacity to produce great amounts of grain with little agronomical practices. The use of this legume, however, is less effective in pigs than in other species, such as ruminants and poultry. This may be related to the types and levels of antinutritional factors present in the seed (Ly 1996; Nava et al 1999). As it very well known, antinutritional factors are various in nature and effect, and many of them are present in legume seeds (Huissman et al 1990; Makkar 1993; D'Mello 1995).

The best known of these above mentioned substances is concanavalia A, a lectin which has been reported to have negative effects on nutrient absorption (León et al 1991). L-canavanine, has also been reported as a non protein amino acid present in canavalia bean N fraction (Rosenthal 1977, 1991). On the other hand, it has also been shown that urease activity and protease inhibitors are characteristics of canavalia beans (D'Mello 1995; Nava 2006).

In spite of the efforts to neutralise the negative aspects of canavalia beans, levels of inclusion even at five percent in diets for pig are not recommended. Among the methods applied to the grain, the most effective have been ensiling and autoclaving, whereas soaking and cooking did not alter the antinutritional factors encountered in canavalia beans. On the other hand extrusion of the product resulted in a low intake of the diet and low daily gains in young pigs. Overall, different attempts to improve the use of canavalia beans in diets for pigs are yet to come successful (Pérez 1997; Nava 2006).

This communication reports some studies concerning the ileal and faecal digestibility and the energy and nitrogen balance of ground and pre-soaked, then autoclaved canavalia beans in pigs.

## MATERIALS AND METHODS

The canavalia beans or jackbeans (*Canavalia ensiformis* L.) were Cuban in origin, and were recently harvested from a farm near the Institute. The methods employed for the detoxification consisted of soaking coarsely ground beans during twenty four hours, washing with water and then autoclaving the soaked washed seed at 120°C during three hours. After autoclaving, the beans were soaked again during another 24 hours, then washed with water and thereafter mixed in fresh state with the other components of the diet. Three diets based on sugar cane molasses type B and soybean meal were formulated to contain graded levels of the treated canavalia beans (0, 5 and 10% in dry basis). The composition of the diets was described elsewhere (Ly and Delgado 2007), and relevant characteristics of its composition are presented in table 1.

Two experiments were conducted to determine digestive indices in pigs fed on canavalia beans containing diets. In the

first experiment six crossbred growing barrows weighing on average 40 kg live weight were allotted at random into three diets according to a duplicate 3 x 3 Latin square design to determine the effect of treated canavalia beans in the diet of pigs. The animals were intact pigs, housed individually during seven days in 1.2 x 2.0 m pens in an open room. Feed was supplied to the pigs at a daily level of 0.08 kg DM/W<sup>0.75</sup>. The daily allotments of feed were divided into two equal meals at 9:00 am and 3:00 pm during the adaptation period. Throughout the experiments, pigs were given free access to water from a nipple drinker. After adaptation to diets, the animals were housed in metabolism cages for the duration of the collection period, which length was of five days following two previous days of adaptation to cages. The cages were equipped with a feeder and a nipple drinker and had expanded metal slatted floors. During the five day collection period, feed intake, including feed refusal was recorded, whereas faeces and urine were quantitatively collected every day. Faeces and an aliquote of daily acidified urine were immediately frozen and stored until analysis.

In the second experiment, three animals of 35 kg body weight and fitted with an end-to-end ileo-rectal anastomosis were distributed at random over the same experimental treatments, a 3 x 3 Latin square, as in the first experiment. Details of the surgical procedure were described elsewhere (Dominguez et al 2000). Following the surgery, pigs were individually housed in the metabolism cages previously described. After an adaptation period of five days, ileal digesta was collected continuously during 48 hours.

**Table 1. Characteristics of the experimental diets (per cent in dry basis)**

Ingredients	Canavalia beans, %		
	-	5	10
Sugar cane molasses B	66.5	63.8	61.1
Soybean meal	31.2	28.3	25.7
Treated canavalia beans <sup>1</sup>	-	5.5	10.8
Macrominerals <sup>2</sup>	2.0	2.0	2.0
Vitamins and minerals <sup>3</sup>	0.3	0.4	0.4
<b>Analysis</b>			
N	2.42	2.46	2.50
Energy, kJ/g DM	15.32	15.40	15.50

<sup>1</sup> For details, see text

<sup>2</sup> Composition of macrominerals was described by Ly et al (2009)

<sup>3</sup> The premix provided the following quantities of vitamins and minerals per kg of complete diet: 27 mg FeSO<sub>4</sub>.7H<sub>2</sub>O, 10 mg MnSO<sub>4</sub>.4H<sub>2</sub>O, 15 mg CuSO<sub>4</sub>.5H<sub>2</sub>O, 85 mg MgSO<sub>4</sub>.7H<sub>2</sub>O, 0.3 mg CoSO<sub>4</sub>.7H<sub>2</sub>O, 0.1 mg KI, 0.02 mg Na<sub>2</sub>SeO<sub>3</sub>, 1 600 IU vitamin A, 300 IU vitamin D<sub>3</sub>, 2 mg thiamine, 3 mg riboflavine, 300 mg choline, 15 mg niacin, 5 mg pantothenic acid, 15 mg pyridoxine, 0.5 mg folic acid, 25 mg cyanocobalamin

Samples of diets, ileal digesta and faeces were analysed by duplicate for nutrient content using standard methods (AOAC 1998). Raw and treated canavalia beans were dried and finely ground prior to chemical analysis. The trypsin inhibitor activity was determined as outlined by Smith et al (1980) and

Hammerstramd et al (1981). L-canavanine was estimated by the colorimetric method suggested by Bell (1958). In the case of urease activity, the procedure of the RMACE (1979) was employed. The nutritive value of the treated canavalia bean used in both designed experiments was calculated by difference following Crampton y Harris (1969).

All data were subjected to analysis of variance according to the Latin square design (Steel et al 1997). In the case of digestibility indices estimated by difference for canavalia beans, means were compared by analysis of variance according to the t test. In all cases, the Harvey (1990) software was employed for data computation.

## RESULTS

The characteristics of raw and autoclaved canavalia beans are shown in table 2. As a result of the treatment trypsin inhibitor activity decreased from 10.1 to 2.5% whereas the successive washings reduced the canavanine content from 2.01 to 0.25%. It is well known that trypsin inhibitors are thermolabile, and that L-canavanine is a free toxic amino acid which is heat-stable, but highly soluble in water.

**Table 2. Composition of canavalia beans (per cent in dry basis)**

	Canavalia beans	
	Raw	Treated
Dry matter	88.15	87.98
Organic matter	96.79	98.00
N x 6.25	33.63	31.19
Gross energy, kJ/g DM	17.58	18.27
L-canavanine	2.01	0.25
Trypsin inhibiting activity <sup>1</sup>	10.1	2.5
Urease activity	30.5	0.5

<sup>1</sup> Expressed as mg/g

<sup>2</sup> Expressed as mg/g per min incubated at 30°C

The pigs stayed healthy throughout the experiment and readily consumed their diets, except in diets containing 10% canavalia beans, where feed refusals were evident in the ileorectostomized pigs, which ate some 96% of the daily allowance. This phenomenon was not observed in the intact animals. Digestibility of dry matter, organic matter, energy and nitrogen from these two trials are summarized in table 3.

**Table 3. Ileal and rectal digestibility in pigs fed diets containing treated canavalia beans**

	Treated canavalia beans, %			SE ±
	-	5	10	
<b>Ileal digestibility, %</b>				
Dry matter	85.7	85.4	84.5	0.6 <sup>+</sup>
Organic matter	88.0	87.3	87.3	0.8 <sup>+</sup>
Energy	87.0	86.3	86.8	0.7
N x 6.25	76.7	75.0	75.0	1.4
<b>Rectal digestibility, %</b>				
Dry matter	92.4	91.6	92.5	0.5 <sup>+</sup>
Organic matter	92.6	91.8	93.0	0.6
Energy	91.6	91.1	91.7	0.9
N x 6.25	84.6	84.8	84.9	1.3

<sup>+</sup> P<0.10

It was observed that energy (y) and organic matter (s) digestibility of the assayed diets were highly correlated (P<0.001) either at the rectum or the distal ileum site, the regression being the following:

$$\text{Distal ileum, } y = 0.104 - 1.107 x \text{ (SE } \pm 0.109; r, 0.961)$$

$$\text{Rectum, } y = 0.0937 + 1.014 x \text{ (SE } \pm 0.955; r, 0.955)$$

According to the results shown in table 4, the energy balance was not affected by treatments, and this indicates that at the level tested, canavalia beans did not appear to exert a negative influence on energy metabolism of the animals. On the other hand, differences in daily feed intake did not reveal any significant (P>0.10) effect, and therefore, no adjustment of data was considered necessary to be conducted. Overall, results derived from the energy balance suggested an efficient utilization of gross energy.

**Table 4. Energy balance in intact pigs fed diets containing treated canavalia beans**

	Treated canavalia beans, %			SE ±
	-	5	10	
<b>n</b>	6	6	6	-
<b>Energy balance, MJ/day</b>				
Consumption	18.95	18.84	16.68	0.42
Faecal output	1.78	1.79	1.37	0.14
Digestion	17.17	17.04	15.31	0.33
Urine output	0.74	0.83	0.61	0.13
Total output	2.52	2.62	1.98	0.16
Retention	16.43	16.22	14.70	0.20
<b>Retention</b>				
As % of intake	86.9	85.7	86.6	0.9
As % of digestion	94.4	94.6	95.3	0.8

Results concerning the nitrogen balance are also shown in table 5. In this particular case, N consumption by animals fed the diet containing 10% seeds appeared to be lower than the other two assayed diets, but this effect was without any significant (P>0.10) consequence.

**Table 5. N balance in intact pigs fed diets containing treated canavalia beans**

	Treated canavalia beans, %			SE ±
	-	5	10	
<b>n</b>	6	6	6	-
<b>N balance, g/day</b>				
Consumption	29.96	30.79	28.87	0.39
Faecal output	4.62	4.68	4.34	0.49
Digestion	25.34	26.11	24.53	0.29
Urine output	8.73	7.43	6.62	0.73
Total output	13.35	12.15	10.96	0.83
Retention	16.61	18.64	17.91	0.70
<b>Retention</b>				
As % of intake	56.1	59.4	62.0	2.4 <sup>+</sup>
As % of digestion	65.4	70.2	73.0	2.8 <sup>+</sup>

<sup>+</sup> P<0.10

The N balance tended (P<0.10) to be increased with an increasing level of canavalia beans in the diet. Indeed, 10% of

canavalia bean introduced in the diet determined that N retention was 10.5% and 11.6% higher than the control, when expressed either as percentage of that consumed or of that digested.

The nutritive value for pigs of canavalia beans, as calculated by difference, did not reveal to be affected ( $P>0.10$ ) by the level of introduction of beans in the diet of the animals, at least up to 10% (table 6), either at the ileal or at the rectal site of pigs. In fact, no differences were observed for DM, organic matter, N and energy digestibility in the treated beans used in the current investigation.

**Table 6. Nutritive value of treated canavalia beans for pigs<sup>1</sup>**

	Digestibility, %		
	5	10	SE ±
<b>Up to ileum (n = 3)</b>			
DM	79.6	73.7	3.5
Organic matter	74.0	74.0	3.5
Energy	73.0	69.0	3.0
N	42.6	59.7	8.5
<b>Up to faeces (n = 6)</b>			
DM	80.4	3.4	6.1
Organic matter	76.6	96.6	8.9
Energy	81.6	92.6	5.5
N	58.2	73.2	8.0

<sup>1</sup> Estimated by difference

Since the absence of significant differences in bean nutritive value as a consequence of the level of inclusion of canavalia beans in the diet was evident, the estimations of ileal and rectal digestibility of the seeds were pooled.

The resulting values are listed in table 7. As to be expected from nutrient digestibility observed in the experimental diets, organic matter digestibility of canavalia beans was relatively high, 74.1 and 86.6% up to ileum and up to faeces, respectively. In this connection, ileal organic matter digestibility accounted for 85.5% of total or rectal organic matter digestibility. N digestibility was not high, in this opportunity, 51.2% of canavalia bean N which was digested in the pigs, disappeared before digesta reached the caecum of animals. On the other hand, rectal N digestibility of canavalia beans was relatively low too, accounting for 65.7% at the rectum level.

**Table 7. Nutritive value of treated canavalia beans for pigs**

	Value <sup>1</sup>
<b>Ileal digestibility, %</b>	
DM	76.7 ± 3.1
Organic matter	74.0 ± 3.2
Energy	71.0 ± 2.8
N	51.2 ± 7.0
<b>Rectal digestibility, %</b>	
DM	86.9 ± 5.5
Organic matter	86.6 ± 7.0
Energy	87.1 ± 5.4
N	65.7 ± 7.1

<sup>1</sup> Mean and standard error of 3 and 6 determinations in ileal and rectal sites, respectively

## DISCUSSION

Due to feed consumption inhibition as probably due to L-canavanine presence in canavalia beans (Enneking et al 1992; Nava et al 2000) and eventually death of animals (Moncada et al 1986) as caused by concanavalin A activity too, very few information is available concerning data of digestibility indices in pigs (Pérez 1997; Yin et al 1994, 2002). According to results of the present investigation, the inclusion of ground, soaked, washed and autoclaved seeds up to 10% in diets for pigs, only has a slight negative effect on apparent ileal digestibility of dry matter, organic matter and energy. On the other hand, the energy balance did not appear to be affected by the inclusion of the autoclaved canavalia beans if included up to 10% in the diet. Besides, high values for digestion and metabolism of energy is common to be observed in pigs fed on enriched sugar cane molasses (Ly 2008).

Ileal N digestibility data, as it was found in the current study, are in the middle of the range of values observed in other laboratories where the nutritive value of canavalia beans was examined. On the other hand, the data herein reported are in agreement with others from a batch of this type of seed treated by the same procedure described above, when an in vitro (pepsin/pancreatin) procedure was employed (Ly and Delgado 2007). Pérez (2007) provided information involving growing pigs fed canavalia beans which were toasted at 194°C for 18 min and thereafter introduced the seeds up to 20% in conventional diets based on cereals and grains. In this connection, Pérez (1887) found that ileal and rectal digestibility of N in the canavalia beans he used, accounted for some 30.9 and 42.0% respectively. In contrast, it has been found that ileal N digestibility was not so drastically depressed in pigs fed on canavalia beans (Yin et al 1994, 2002). For example, in the experiment of Yin et al (2002), the seeds included at a level of 55% in a semi-synthetic dietary formula, determined an apparent ileal digestibility of N as high as 70.2%. These same authors encountered that canavalia beans enveloped in nylon bags had a digestibility of N equal to 71.9%, when the bags were analyzed following collection from the terminal ileum and washing it during two minutes. It is not known the status of the canavalia beans used by Yin et al (2002), which had higher N digestibility than the seeds herein evaluated.

It is difficult to determine the nature of disparities found among results related to N utilization by pigs fed treated canavalia beans. In this connection, the difference above mentioned could be explained on one hand, by certain positive influence of the energy source employed in the experimental diets, since in the current evaluation a high proportion of sugar cane molasses based diets (Ly 2008), or on the other hand, a remarkable effect of the method of neutralization of anti-nutritional factors of canavalia beans. Overall, it has been claimed that incomplete suppression of anti-nutritional factors, such as lectins, during treatment of legume seeds conduces to a noteworthy decrease in ileal digestibility of N due to excessive endogenous N losses (see for example, Van der Poel and Huisman 1988). Data from a companion paper concerning ileal output of digesta in pigs fed canavalia beans are in accordance with these findings (Ly et al 2009). In any way, drastic heat treatment of seeds may involve undesirable, negative side effects on N utilization, such as the presence of products from a Maillard reaction, as it was suggested by Ly and Delgado (2007).

Nitrogen balance appeared to be favourably influenced by 10% of treated canavalia beans in the diet. This could be caused by some changes in the urea cycle activity due to the remaining L-canavanine found in the seeds. As it is known, L-canavanine is an amino acid isomer of arginine and there are evidences that L-canavanine blocks the urea cycle effectively through competitive inhibition (Rosenthal 1977, 1991). Nevertheless, a low ileal digestibility of N in this legume grain, as observed in the current investigation, could be linked to N endogenous losses at the ileal level, such as it was reported in a companion paper (Ly et al 2009), and methods to overcome this constraint should be assessed.

It is evident that more research should be conducted from the point of view of practical methods to inactivate toxic substances from canavalia beans, since autoclaving is an expensive method of heat treatment. Nevertheless, other attempts to detoxify these seeds, in order to stimulate feed consumption and digestion of canavalia beans, such as alkali treating, roasting or even extruding (Risso and Montilla 1992), boiling (Belmar and Morris 1994), toasting (Pérez 1997) and ensiling (Nava and Belmar 1999; Nava 2006) have revealed to be unsuccessful, at least in the manner treatments were conducted.

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