

Revista Electrónica Nova Scientia

Protein and energy evaluation of detoxified
Canavalia seeds as a feedstuff for poultry in the
tropics

Evaluación proteínica y energética de semillas
tratadas de *Canavalia* empleadas como alimento
para aves en el trópico

**Luis Antonio Chel-Guerrero¹, América Delgado-Herrera²,
David Abram Betancur-Ancona¹, Patricia Pérez-Aviña² and
Arturo Francisco Castellanos-Ruelas¹**

¹Facultad de Ingeniería Química, *Campus* de Ingenierías y Ciencias Exactas,
Universidad Autónoma de Yucatán, Mérida

²Former student of the graduate program

México

A.F. Castellanos-Ruelas. E-mail: cruelas@correo.uady.mx

Resumen

Las leguminosas son un recurso proteínico muy importante en las zonas tropicales, tanto para alimentación humana, como animal; entre ellas la *Canavalia ensiformis* se utiliza ampliamente en muchas partes del mundo. El inconveniente más importante de su empleo es la presencia de factores anti-nutricionales tales como fitatos, glúcidos cianogénicos, taninos, entre otros. Varios tratamientos con calor permiten eliminar algunos de estos tóxicos, pero las altas temperaturas reducen la digestibilidad de la proteína y la concentración de energía metabolizable. El tratamiento mediante el empleo de calor reducido junto con solubilización por salado o bien, la separación de la fracción de proteína del resto de los componentes del grano, pueden ser métodos alternativos para eliminar los elementos tóxicos. El objetivo de este trabajo fue disminuir los factores anti-nutricionales de *Canavalia ensiformis* tratándola con calor ligero combinado con solubilización por salado o mediante la eliminación de la fracción tóxica obteniendo un concentrado proteínico, midiendo el posible cambio en la digestibilidad de la proteína y el valor de la energía metabolizable verdadera, con la finalidad de incorporarla en dietas para aves. Los frijoles fueron molidos y sometidos a dos tratamientos experimentales y uno testigo: eliminación de factores tóxicos utilizando el fenómeno de solubilización por salado y posteriormente aplicando calor ligero (ASOL-H); y la extracción de concentrado proteínico (PC). Como tratamiento testigo (T) se empleó la *Canavalia* cruda sin tratamiento. Se analizó el contenido de las siguientes variables: fitatos, glúcidos cianogénicos, taninos, inhibidores de tripsina, lectinas y canavanina, así como la digestibilidad proteínica y la energía metabolizable verdadera. Los resultados obtenidos fueron interpretados calculando la media y desviación estándar; también se analizaron mediante el procedimiento de Modelos Lineales Generales (GLM) para detectar efectos de los tratamientos (ASOL-H, PC y T) sobre las variables estudiadas. Los resultados mostraron efecto de los tratamientos ($P < 0.05$) sobre las variables, reduciendo el contenido de tóxicos. PC redujo de manera más eficiente todos los compuestos tóxicos. La digestibilidad de la proteína y la energía metabolizable verdadera no se afectaron por el uso de los tratamientos utilizados. El uso de ambos tratamientos, pero especialmente PC, es recomendable para eliminar la toxicidad sin afectar el valor de la proteína y la energía de *Canavalia* con la finalidad de utilizarla en dietas para aves.

Palabras clave: Factores antinutricios; digestibilidad de proteína; energía metabólica neta.

*Recepción: 05-07-2016**Aceptación: 20-09-2016*

Abstract

Legumes are a very important protein resource in tropical areas both for human and animal consumption. Among them, *Canavalia ensiformis* is used widely in many parts of the world. The most important inconvenience of the use of *Canavalia* is the content of anti-nutritional factors such as phytates, cyanogenic glycosides and tannins among others. Various treatments using heat are available to eliminate toxicity, but high temperature reduces protein digestibility and energy concentration. Treatments using low heat in combination with salting or the separation of the protein fraction from the rest of the constituents of the grain, may be alternative methods to eliminate toxic elements. The objective of these trials was to reduce the antinutritional factors and to determine the possible change in protein digestibility and TME content of *Canavalia ensiformis* after detoxification using low heat combined with salting-in or obtaining a protein concentrate, in order to incorporate it into poultry diets. Beans were grinded and submitted to two different experimental treatments and control: Detoxification using an acidified saline solution with low heat (ASOL-H); and an extraction of its protein concentrate (PC); raw *Canavalia* was used as Control. The following toxic compounds were analyzed: phytates, cyanogenic glycosides, tannins, trypsin inhibitors, lectins and canavanine, as well as protein digestibility and true metabolic energy. Means and standard deviation were calculated and data was also analyzed using the General Lineal Model (GLM) procedure to detect effects of treatments. Results showed a sharp reduction of antinutritional factors due to treatments ($P>0.05$). PC reduced more efficiently all toxic substances. Protein digestibility and TME were not affected by the use of either method of detoxification. Therefore the use of both treatments, but specially PC, may be advantageous to eliminate toxicity preserving the protein and energy value of *Canavalia* to be used in poultry diets.

Keywords: antinutritional factors; protein digestibility, true metabolic energy.

Introduction

Canavalia ensiformis is a legume high in protein also known as Jack bean, Snake bean, Horse bean, White bean, Creole bean. It is scattered in the tropics and subtropics across the world, but it is not cultivated on a large scale.

It is a promising crop in Yucatán, México, since in an agronomical trial on stony soil (cultivated seasonally, without fertilization) a maximum yield of 1.9 tons / ha was obtained, which is more than the obtained with corn or *Phaseolus vulgaris* under the same conditions (Herrera, 1983). This makes it an alternative as a feedstuff for farm animals.

The utilization of raw *Canavalia*, as any other legume, is risky since it has antinutritional components limiting its use as a feed ingredient for humans and livestock, particularly monogastrics. Besides having non absorbable phosphorous in form of phytic acid, *Canavalia* seeds have cyanogenic glycosides, tannins, proteases inhibitors, lectins, L-canavanine, urease, concanavalin A and B (Rajaram and Janardhanan, 1992), among other toxic substances. When *Canavalia* is administered raw, representing between 250 to 500 g / kg in the diet of broilers, serious tissue damage is observed in pancreas, lungs, intestines, liver and kidney (Ologhobo *et al.*, , 2003).

Some available methods to eliminate toxicity are: soaking and heating (3 h at 95° C) the broken kernels (Udedibie and Carlini, 1998); cooking at 100°C for 50 min (Alagbaoso *et al.*, 2015); roasting at 190 °C or more during 16 min (Mendez *et al.*, 1998; Michelangeli *et al.*, 2004); steaming at 120 psi during 45 min (Akande *et al.*, 2013) among others. Another treatment using heat between 180-200 °C, eliminates effectively the antinutritional factors in raw *Canavalia* and therefore enhances the energy value of the seed to 2.4 Mcal ME/kg (Síboli *et al.*, 2004), but this amount of energy is considered to be low. The success of all this treatments is based on the fact that most of this antinutritional factors are thermolabile; but some are thermostable like saponins and may render inefficient heat treatments and could help to explain the lack of success in trials such as one carried out with fattening pigs (Emenike *et al.*, 2016). In most of this experiments no information on protein quality value was investigated. Since the use of high heat may reduce indirectly the protein digestibility of a feedstuff, as well as the metabolic energy content due to Maillard reactions (Payle and Gerrard, 2002), it would be expected that a detoxifying method using low heat or no heat, may be more suitable to preserve the nutritional value of *Canavalia*.

There are very few methods that use low temperature or do not use temperature at all. The use of low heat in an extraction using an acidified saline solution proved to be efficient removing most of the antinutritional components of *C. ensiformis* (Gamboa *et al.*, 1994): this method is simple to achieve as well as inexpensive. Another method that does not use temperature and also eliminated toxic substances of *Canavalia* is the extraction of a protein concentrate (Betancur-Ancona *et al.*, 2008), but no information concerning the protein digestibility and the metabolic energy content was provided in these reports either. Therefore the objective of the present work was to reduce the antinutritional factors and to determine the possible change in protein digestibility and True metabolic energy content of *Canavalia ensiformis* after detoxification using low heat or obtaining a protein concentrate, in order to incorporate it into poultry diets.

Materials and methods

Fifty kg of *Canavalia ensiformis* obtained from X'pujil community located in the state of Campeche, were used. Beans were grinded and submitted to two detoxification methods: treated in an acidified saline solution (salting-in phenomena) submitted to low heat (ASOL-H); the extraction of a protein concentrate (PC). Two experiments were carried out:

Experiment 1

To obtain the ASOL-H flour, a dispersion of 5 kg of *Canavalia* was prepared in 25 L of 0.5 M NaCl solution at a 1: 5 (w / v) (salting-in). The pH was adjusted to 4.9 with 2N HCl, the mixture was heated and stirred at 80°C for 60 min. The suspension was cooled at room temperature and then centrifuged at 2500 rpm for 10 min at 25°C in a Mistral centrifuge model 3000i (Sanyo-Gallen Kamp, Leicester, UK). The supernatant was discarded and the precipitate dried at 60° C for 12 h. ASOL-H obtained was weighed and ground in a mill through a mesh 20 grid.

The PC was obtained using the method described by Betancur *et al.*, (2004) which consists in a humid fractionation of grinded seeds, alkaline solubilization and precipitation of protein at isoelectric point. After the elimination of the supernatant, the precipitate was dried. The method was as following: a dispersion of 10 kg of *Canavalia* flour in 70 L water (1: 7 w / v) was prepared. The pH was adjusted to 12 with 2N NaOH and allowed to stand 12 h at room temperature. Subsequently the suspension was sieved through 80 mesh grid (0.177 mm pore opening), to separate the solid with the fiber and the liquid with the protein and starch. The fiber residue was discarded. The supernatant

was passed through a 150 mesh grid (0.106 mm pore opening) to remove the finest fiber. The suspension was allowed to stand for 2 h until complete sedimentation of starch. Solubilized protein in the supernatant was siphoned.

Proximal analysis in raw, ASOL-H and PC were carried out using A.O.A.C. (2012) methods. Antinutritional compounds were also estimated. A synthetic description of the methodologies utilized is described.

Total phytates (Makkar *et al.*, 2007). Extraction was carried out with 3.5% (w/v) HCl and further purified through an AG1-X8 chloride anion exchange column. The pink color of Wade reagent, previously prepared, was due to the reaction between ferric ion and sulfosalicylic acid with an absorbance maximum at 500 nm. In the presence of phytate, the iron became bound to the phosphate ester and was unavailable to react with sulfosalicylic acid, resulting in a decrease in pink color intensity

Cyanogenic glycosides (A.O.A.C., 1990). Cyanide was distilled from a chloroform and water solution into KOH solution, forming KCN which was titrated with silver nitrate; $2\text{KCN} + \text{AgNO}_3 = \text{KCN} \cdot \text{AgCN} + \text{KNO}_3$. An excess of AgNO_3 produced insoluble AgCN , which was the end point of the titration: $\text{Ag}(\text{CN})_2\text{K} + \text{AgNO}_3 = 2\text{AgCN} + \text{KNO}_3$

Tannins (ISO-9648, 1988). The method consists in their extraction by shaking with dimethylformamide, then after centrifuging, addition of ferric ammonium citrate and ammonia to an aliquot part of the supernatant liquid and spectrometric determination, at 525 nm, of the absorbance of the solution thus obtained, and determining of the tannic content using a calibration curve prepared using tannic acid. This technique measures total phenols and tannins.

Trypsin inhibitors activity (Kakade *et al.*, 1974). It was measured using BAPNA (Benzoyl-L-arginine-D-p-nitroanilide) as a substrate and the results reported as units of trypsin inhibited (UTI). A trypsin unit (UT) is defined as the increase in 0.01 absorbance units at 420 nm per 5 ml reaction mixture under the experimental conditions.

Lectins (Jaffe *et al.*, 1974). Hamster red blood cells were used, washed and sensibilized with trypsin using a microdilutor, to take into account the number of times that the samples were diluted.

Canavanine (Makkar *et al.*, 2007). The guanidoxy group, $-\text{O} \cdot \text{NH} \cdot \text{C} (: \text{NH}) \cdot \text{NH}_2$, in canavanine reacted with trisodium pentacyanoammonioferrate in aqueous solution at pH 7.0 and formed a magenta-colored chromophore. The color formed was measured at 520 to 530 nm using

as a reference a standard curve with canavanine (reagent grade) in order to calibrate the spectrophotometer.

Experiment 2

Protein digestibility was evaluate in ASOL-H and PC using the multienzyme method described by Hsu *et al.*, (1977).

Finally, a bioassay was carried out to estimate the true metabolic energy value (TME) of *Canavalia* using nine Leghorn roosters (Sibbald, 1976). The TME was estimated subtracting the excreted energy to the consumed energy. Treatments were as follow: experimental diet with ASOL-H, incorporated in the diet at 25, 30 and 35% of the protein requirements of the birds (NRC, 1994). Experimental diet with PC, incorporated the same way as the previous one. Control diet. All diets were made with ground corn, soybean meal, wheat bran, vegetable oil and they were added with premix of vitamins and minerals, calcium carbonate, Ca orthophosphate, lysine, methionine and salt, to meet the nutritional requirements of growing broilers (NRC, 1994).

Statistical analysis

Means and standard deviation of data were estimated in both experiments. One-way ANOVA method was applied to verify for significant differences between the treatments where applicable, followed by Tukey's Test (SAS, 1988).

TME value was analyzed according to a completely randomized design by means of two Latin Squares 3x3, one for ASOL-H and another for PC, each one with three replicates. The model was:

$$Y_{ij(k)} = \mu + \text{Row}_i (\text{animals}) + \text{Col}_j (\text{periods}) + t_{(k)} (\text{treatments}) + E_{ij(k)} (\text{random error})$$

Analyses were performed with the Means, ANOVA and GLM routines of the SAS statistical software (SAS, 1988).

Results

Experiment 1

Analysis of chemical composition of raw and treated *Canavalia* seeds are shown in Table 1. Treated ASOL-H had a lower amount of protein ($P < 0.05$) compared to raw *Canavalia* since some

part of it (albumin) is water soluble. PC obviously had the highest amount of protein ($P<0.01$). Changes in all other constituents fluctuated as expected.

Table 1. Chemical composition of raw (RC) and treated *Canavalia* flour (% DM) ($n=3$)^{1,2}

ANALYSIS	RC	ASOL-H	PC
Moisture	10.2 ^a ± 0.2	9.8 ^a ± 0.1	6.7 ^b ± 0.1
Crude protein	31.4 ^a ± 0.1	24.2 ^b ± 0.1	79.0 ^c ± 0.1
Ether extract	2.6 ^a ± 0.1	2.3 ^a ± 0.0	4.4 ^b ± 0.1
Crude fibre	6.6 ^a ± 0.1	2.0 ^b ± 0.0	0.5 ^c ± 0.1
Ash	5.8 ^a ± 0.1	6.0 ^a ± 0.2	6.0 ^a ± 0.1
Nitrogen free extract	53.1 ^a ± 0.1	65.0 ^b ± 0.1	9.6 ^c ± 0.1

¹Mean ± SD; ASOL-H= Treated *Canavalia* in a salty-acid solution submitted to heat; PC= *Canavalia* protein concentrate.

²Different letters in the same line represent a-b= $P<0.05$, a-c= $P<0.01$.

Table 2 shows the analysis of antinutritional factors in raw and treated *Canavalia*. A significant reduction in all the antinutritional factors was obtained with the treatments used. Maximum reduction ($P<0.01$) was observed with cyanogenic glycosides, trypsin inhibitors, Canavanine and lectins. A minor effect, but also important was obtained with phytates and tannins.

Table 2. Antinutritional factors in raw (RC) and treated *Canavalia* flour (%DM) ¹

ANALYSIS	RC	ASOL-H	PC
Phytates (mg/g)	3.62 ^a ± 0.01	2.06 ^b ± 0.02	1.87 ^c ± 0.01
Cyanogenic glycosides (mg%HCN)	3.02 ^a ± 0.50	0 ± 0	0.77 ^c ± 0.04
Tannins (% Tanic acid)	0.26 ^a ± 0.02	0.19 ^b ± 0.01	0.16 ^c ± 0.03
Trypsin inhibitors (UIT/mg) ³	26.04 ^a ± 0.01	4.08 ^c ± 0.04	2.06 ^c ± 0.04
Lectins (HU) ⁴	33 ^a	32 ^a	4 ^c
Canavanine g/100g of protein	14.3 ^a	0.23 ^c	0.16 ^c

¹Mean ± SD; ASOL-H= Treated *Canavalia* in a salty-acid solution submitted to heat; PC= *Canavalia* Protein concentrate.

²Different letters in the same line represent a-b= $P<0.05$, a-c= $P<0.01$.

³UIT. Units of inhibited trypsin.

⁴HU. Haemagglutinating units

Experiment 2

Results on the protein quality and energy value of *Canavalia* are shown in Table 3. Protein digestibility was improved in PC ($P<0.05$) compared to raw *Canavalia*; no changes were observed in ASOL-H compared to raw *Canavalia*. No statistical difference was detected in the energy content between raw vs treated seeds ($P>0.05$).

Table 3. Protein digestibility and energy value of *Canavalia ensiformis* depending on treatments (n=9)^{1,2}

ANALYSIS	Raw	ASOL-H	PC
<i>Canavalia</i>			
Protein digestibility	73.8 ^a	74.4 ^a	82.7 ^b
Mcal TME/ kg DM	3.140 ^a ±0.225	2.895 ^a ±0.425	2.999 ^a ±0.248

¹Mean ± SD; Control= Corn-soybean meal based diet; ASOL-H= Treated *Canavalia* in a salty-acid solution submitted to heat; PC= *Canavalia* protein concentrate.

²Different letters in the same line represents $P<0.05$.

Discussion

Raw *Canavalia* seeds had a high proportion of crude protein (31.4 %, Table 1), similar to grains analyzed by Bramiro (1994) (29.7 %). Such an elevated protein content of the grains used in this experiments was also observed in the PC (79.0%). When the protein recovery out of the total protein content in the grain is above 70%, the extraction method is considered to be efficient (Cantoral *et al.*, 1995), therefore offers a possible economic success if the method is escalated. The percentage of nitrogen free extract had the exact opposite tendency to the one observed with crude protein. All other constituents did not show important changes, except crude fiber, which as expected, was elevated in raw *Canavalia*. Some authors have found a potential use of these fiber residues in the food industry as a functional ingredient in reduced-calorie food (Segura *et al.*, 2014).

The reduction in antinutrients as a percentage showed better efficiency in PC compared to ASOL-H (Table 2). Reduction was very efficient ($P<0.01$) in canavanine (99 % and 98 %), trypsin inhibitors (92 % and 84 %) and cyanogenic glycosides (74 % and 100 %) for PC and ASOL-H respectively. Also efficient, but in a lower degree ($P<0.05$) were pythates (48 % and 43 %) and tannins (38 % and 27 %) for PC and ASOL-H respectively. Finally lectins were only reduced significantly ($P<0.01$) in PC treatment.

Roasting, boiling and fermentation are other alternative methods used to reduce antinutritional factors of *Canavalia*, providing different efficiencies. According to Ajeigbe *et al.*, (2012) mild roasting even increases phytate concentration but boiling and fermentation reduce drastically cyanide (87 % and 88 %) and tannins (93 % and 92 %) respectively. Comparing this results with the present study, ASOL-H and PC treatments where more efficient to eliminate phytates (34% and 48%), cyanide (100 % and 74 %) but less efficient to reduce tannins (27 % and 38 %) respectively.

The use of soaking and autoclaving of raw *Canavalia* (Sasipriya and Sidhuraju, 2013) is less efficient to reduce the toxic non proteic α -aminoacid canavanine (61%), compared to ASOL-H and PC, where reductions of 98% and 99%, respectively were reached. Trypsin inhibitors were efficiently removed by all three methods: PC, soaking and autoclaving: 92 %, 84 % and 99 % respectively.

Previous reports indicate that toasting *Canavalia* at 190°C during 16 min reduced significantly the amount of Concanavaline A (Con-A), a lectin found in *Canavalia*; broiler chicks may consume up to 100 mg of Con-A daily with no deleterious effects on growth (Mendez *et al.*, 1998). Lectin reduction in the present study, was only efficient in PC (88%) therefore this treatment may be suitable to be use when *Canavalia* is considered to be an ingredient to poultry diets. Nevertheless lectins nowadays are considered important because of their bio-functionally due to involvement in immune mechanisms, antibacterial and anticancer mediation where their presence has allowed the explanation of mechanisms of action at a molecular level (Singh and Sarathi, 2012). Therefore, a better way to comprehend the use of this molecule is necessary in order to incorporate it into poultry diets.

Protein digestibility was increased ($P < 0.05$) in the PC in comparisons to raw *Canavalia* where ASOL-H did not showed any deleterious effect ($P > 0.05$) (Table 3). Similar results have been found by other authors, as for example Ly *et al.*, (2015) who reported that soaking *Canavalia* in water during 24 h and then autoclaving it during 60 min increased the *in vitro* N digestibility from 72.1% to 89.1%. Preserving protein quality is very important, since it may have a positive impact on animal performance. The use of toasting at increasing temperatures of more than 200°C, reduced protein solubility of *Canavalia* as it may be expected when high temperatures are used (Pizzani *et al.*, 2006).

The TME value of *Canavalia* was not affected by treatments ($P>0.05$) (Table 3), but this statistical approach must be taken carefully. There was an arithmetical difference of 0.245 Mcal between Raw *Canavalia* and ASOL-H; such a difference was only about half (0.141 Mcal) between Raw *Canavalia* and PC. This difference of 0.245 Mcal is important in poultry diets since it may improve substantially the productivity in growth rate (NRC, 1994). Energy content was found similar or higher in PC (2.999 Mcal) compared to other conventional ingredients of poultry diets, such as fish menhaden (2.977) (NRC, 1994), or peanuts kernels (2.462) (NRC, 1994); but not as elevated as in corn (3.470) (NRC, 1994) a classical energetic source for poultry. Both treatments tested, ASOL-H and PC, rendered higher energy values (2.895 and 2.0999 Mcal respectively) than the one obtained by Siboli *et al.*, (2004) with birds (2.536 Mcal). Other authors (Fagbenro *et al.*, 2004) have found no change in the energy value of *Canavalia* fed to Nile tilapia by means of the elimination of antinutrients cooking cracked seeds at 100°C for one hour. Therefore animal specie may be important to be considered when *Canavalia* is detoxified.

Conclusions

Detoxification of *Canavalia ensiformis* may be partially achieved treating it with a solution of salt and acid with low heat; most antinutritional factors were reduced 27 % or more, except lectins. The extraction of a *Canavalia* protein concentrate eliminates more efficiently all these toxic agents. Protein digestibility was improved in the protein concentrate and true metabolic energy value was not negatively affected by the use of both methods of detoxification. The reduction of antinutritional factors and the preservation of protein and energy value using either of both treatments, may facilitate the incorporation of *Canavalia* into poultry diets.

References

- Alagbaoso, S.O., Nwosu, J.N., Njoku, N.E., Umelo, M.C., Eluchie, C. and Agunwa, I.M. (2015). Effect of processing on the nutritional and anti-nutritional properties of *Canavalia plagioperma piper* seeds. *European Journal of Food Science and Technology*. 3(3): 45-69.
- Ajeigbe, S. O., Mohammed A. K., Yahaya, I.A., Oyelowo A.O. (2012). Effects of processing techniques on levels of minerals and antinutritional factors of *Canavalia ensiformis*. *Pakistan Journal of Nutrition*. 11(12): 1121-1124.

Akande E. A., Oladipo A. O., Kelani O.S. (2013). Effects of steaming on the physicochemical properties and the cooking time of Jack Beans (*Canavalia ensiformis*). *Journal of Experimental Biology and Agricultural Sciences* 1(6): 420-423.

A.O.A.C. (1990). Official methods of analysis of the Association of Analytical Chemists, 14 th Edition. Washington, D.C. U.S.A.

A.O.A.C. (2012). Official Methods of Analysis. Association of Official Analytical Chemists. 19th Edition. Washington D.C. U.S.A.

Betancur A.D.A., Gallegos T.S., Chel G.L.A. (2004). Wet fractioning of *Phaseolus lunatus* seeds: partial characterization of starch and protein. *Journal of the Science of Food and Agriculture* 84: 1193-1201.

Betancur-Ancona, D. A., Gallegos-Tintoré, S., Delgado-Herrera A., Pérez-Flores V., Castellanos-Ruelas A. F., Chel-Guerrero L. A. (2008). Some physicochemical and antinutritional properties of raw flours and protein isolates from *Mucuna pruriens* (Velvet bean) and *Canavalia ensiformis* (Jack bean). *International Journal of Food Science and Technology* 43(5): 816-823

Bramiro F.O. (1994). Nutritive value of Nigerian Jack Beans (*Canavalia ensiformis*) *Rivista Italiana Sostanze Grasse* 71(8): 421-424.

Cantoral R., Fernández Q. A., Martínez J. A., Macarrulla M. T. (1995). Estudio comparativo de la composición y valor nutritivo de semillas y concentrados de proteína de leguminosas. *Archivos Latinoamericanos de Nutrición* 45(3): 242-248.

Emenike H.I., Udedibie A.B.I. and Emenalom O.O. (2016). Effect of different processed Jackbean (*Canavalia ensiformis*) meals on the performance of grower pigs. *British Journal of Applied Science and Technology*. 13(6): 1-8.

Fagbenro O.A., Adeparusi E.O. and Jimoh W.A. (2004). Nutrients quality of detoxified jackbean (*Canavalia ensiformis* L.) seeds cooked in distilled water or trona solution and evaluation of the meal as substitute for soybean meal in practical diets for Nile tilapia, *Oreochromis niloticus* fingerlings. *Proceedings of the Sixth International Symposium on Tilapia in Aquaculture (ISTA 6)*, pp. 289-300. (R.B. Bolivar, G.C. Mair & K. Fitzsimmons, editors) Manila. Philippines. September 12-16

Gamboa S.M., Osorno M.H. and Chel G.L. (1994). Efecto del proceso de la harina del grano de la leguminosa *C. ensiformis* sobre sus propiedades funcionales y componentes químicos. *BIOTAM*. 6(2): 41-54.

Herrera G.F. (1983). Effect of planting density of the seed yield of *Canavalia ensiformis*. *Tropical Animal Production*. 8: 153-156.

Hsu H. W., Vavak, D. L., Satterlee L. D., Miller, G. A. (1977). A multienzyme technique for estimating protein digestibility. *Journal of Food Science* 42(5): 1269-1271.

ISO-9648. (1988). Determination of tannin content.

http://www.iso.org/iso/catalogue_detail.htm?csnumber=17487.

Kakade M. O., Rackis J. J., Mc Ghee J. E., Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: A collaborative analysis of improved procedure. *Cereal Chemistry*. 51:376-382.

Jaffé W. G., Levy A., González D. I. (1974). Isolation and partial characterization of bean phytahemagglutinins. *Phytochemistry* 13:2685-2693.

Ly J., Delgado E., Nava R., Belmar R., Reyes Z., Domínguez P.L. (2015). Ileal in vitro (pepsin/pancreatin) and in vivo digestion of treated canavalia (*Canavalia ensiformis*) beans given to growing pigs. *Revista Computadorizada de Producción Porcina* 22(2): 90-93.

Makkar, H. P. S., Siddhuraju P., Becker K. (2007). Plant Secondary Metabolites. In: J.M. Walker (ed), *Methods in Molecular Biology* (1st ed), (New Jersey; Humana). Pp.1–100.

Mendez A., Vargas R. E., Michelangeli C. (1988). Effects of concaavalin A, fed as a constituent of Jack bean (*Canavalia ensiformis* L.) seeds, on the humoral immune response and performance of broiler chickens. *Poultry Science* 77(2): 282-289.

Michelangeli C., Pérez G., Méndez A., Sívol, L. (2004). Efecto del tostado del grano de *Canavalia ensiformis* sobre el comportamiento productivo de cerdos en crecimiento. *Zootecnia Tropical* 22(1): 87-100.

Moguel OY, Betancur AD, Chel GL (1996). Aprovechamiento integral del grano de *Canavalia ensiformis*: extracción de proteína y almión. *Tecnología de Alimentos*. 31(1): 1-16.

NRC. (1994). *Nutrient Requirements of Poultry: Ninth Revised Edition*. National Academy Press. Washington DC. USA.

Ologhobo A., Mosenthin R., Alaka O. O. (2003). Histological alterations in the internal organs of growing chicks from feeding raw Jack Bean or Lima bean seeds. *Veterinary and Human Toxicology* 45(1): 10-13.

Pizzani P., Vargas R. E., Pérez S., Méndez A., Michelangeli C., Sívoli L. (2006). Efectos del tostado sobre el valor de energía metabolizable verdadera y el contenido de factores antinutricionales de harinas de granos de *Canavalia ensiformis* (L.) Revista Científica. Facultad de Ciencias Veterinarias Luz 15(5): 523-530.

Rajaram N., Janardhanan K. (1992). Nutritional and chemical evaluation of raw seeds of *Canavalia gladiata* (Jacq) DC. and *C. ensiformis* DC: The underutilized food and fodder crops in India. *Plant Foods for Human Nutrition* 42(4): 329-336.

SAS. (1988). SAS/STAT. User's Guide: Statistics (version 6.03). SAS Institute Inc. Cary, NC, USA.

Sasipriya G., Siddhuraju P. (2013). Evaluation of growth performance, serum biochemistry and haematological parameters on broiler birds fed with raw and processed samples of *Entada scandens*, *Canavalia gladiata* and *Canavalia ensiformis* seed meal as an alternative protein source. *Tropical Animal Health and Production* 45(3): 811-820.

Segura C. M. R., Manrique R. L., Chel G. L. A., Betancur A. D. (2014). Fiber residues from *Canavalia ensiformis* L., seeds with potential use in food industry. *Agricultural Science* 5: 1227-1236.

Sibbald I. R. (1976). Determination of true metabolic energy in feeding stuffs. *Poultry Science* 55(1): 303-306.

Singh. J., Sarathi B. P. (2012). Non-nutritive bioactive compounds in pulses and their impact on human health: An Overview. *Food and Nutrition Sciences* 3, 1664-1672.

Sívoli, L., Michelangeli. C., Méndez A. (2004). Efecto combinado de la deshidratación en doble tambor y del tostado sobre la Energía Metabolizable Verdadera y factores antinutricionales de harinas de *Canavalia ensiformis*. *Zootecnia Tropical*, 22(3): 241-249.

Udedibie A. B. I., Carlini C. R. (1988). Questions and answers to edible problem of the *Canavalia ensiformis* seed- A review. *Animal Feed Science and Technology* 74(2), 95-106.