

Review Article

Adverse Effects of Anti-nutritive Constituents of Unprocessed Jack Bean (*Canavalia ensiformis*) (L) (DC) Seed Meal on Poultry Performance.

ABSTRACT

Jack bean (*Canavalia ensiformis*) is a good source of plant protein as well as a starch storing seed and on the basis of the apparent nutrient content, it should be an extremely useful ingredient in animal feeds. The use of untreated *Canavalia ensiformis* seed as a feedstuff for poultry is restricted due to the presence of anti-nutritional factors, as is the case with the other tropical legumes. The anti-nutritional constituents of *Canavalia ensiformis* have been reported to include trypsin inhibitors and concanavalin A which are heat-labile and canavanine and canaline which are hydrosoluble. Other antinutrients are saponins, cyanogenic glycosides and phenols. *Canavalia ensiformis* seeds can be used as an animal feed ingredient, since they are good source of starch and protein. However, in order to be used as animal feed ingredient, *Canavalia ensiformis* seeds would have to be thermally processed. Heat processing is universally accepted as an effective means of inactivating most, if not all of the heat-labile toxic constituents of legume grains. Research has showed that dietary inclusion of detoxified jack bean seed between 20-30% in feed ration is capable of supporting the growth of poultry birds without adverse effects on performance and physiological parameters of the animals. Several research works had been carried out on how to improve the quality of legumes. These research workers have reported many processing methods. Interestingly, on many occasions, in order to achieve complete inactivation or detoxification of these anti-nutritional factors a combination of two or more processing methods may be needed.

Keywords: *Canavalia ensiformis*, trypsin inhibitors, concanavalin A, canavanine, canaline, saponins, cyanogenic glycosides, poultry

1. INTRODUCTION

Jack bean (*Canavalia ensiformis*) has a great potential as an economic crop because of its excellent germination and rigorous initial growth, which makes its establishment relatively easy [64]. The crude protein content of dry ripe jack bean seed ranges from 26 – 32%. [62] Stated that raw jack bean seeds contain about 30% crude protein and 60% nitrogen free extract. [2] Presented the proximal composition of jack bean as containing 28.38% and 28.90% crude protein, 3.10% and 2.72% crude fat and nitrogen free extract as 53.62% and 51.41% for processed and raw jack bean seeds respectively. The proximate composition of jack bean as shown in Table 1 reveals its potential value for future use in livestock feeding. [10] Reported that jack bean contained about 1-8% ether extract, 8.5% crude fibre, 3.2% ash, 13.5% moisture, 26% crude protein and 46.1% carbohydrate. [17] Reported 2.12%, 2.8% and 9.24% for ether extract, ash and crude fibre, respectively. Studies by [60] showed that jack bean contains about 3.16% ether extract, 3.73% ash and 7.8% crude fibre. The ether extract and ash contents of jack bean seeds are quite low while the crude fibre is slightly high. The gross energy content of 4.26kcal/g for raw jack bean seeds was reported by [60] for raw jack bean seed.

Jack bean seed is relatively low in the sulphur amino acids, methionine, but high in lysine. It contains significant amounts of thiamine, niacin, phosphorus, calcium and iron ([10]; [17]; [33]; [61]).

The use of untreated (raw) seeds of jack beans (*Canavalia ensiformis*) in diets of livestock animals is restricted by the presence of various factors which are toxic. However, properly processed jack bean seeds, an unconventional feedstuff have great potential for inclusion in animal diets. Generally, unconventional feed ingredients are very useful for compounding animal feed in geographical locations or in a situation or season when other sources of vegetable protein (soybeans, groundnut cake, and cotton seed meal) are either not available or are too expensive ([4]; [22]; [44]).

When considering anti-nutritive substances in the seeds of jack bean which may serve as potential feedstuff for livestock, it is worth noting that it is particularly the toxic effect connected with dietary intake of the factor that is significant nutritionally, and not the injection of the antinutrient into the body. Due to the fact that ingested substances may subjected to possible modification by the gastro-intestinal tract ([13]).

Not a sentence.

It has been reported by [10] that both wet and dry thermal treatment resulted in beneficial effect on protein quality with protein efficiency ratio (PER) increasing from raw seed value of 0.10 to 1.21 and 1.18 for wet and dry cooking. These workers also reported the protein quality to be 41.5% as compared with that of casein. The value is similar to that of common food legumes ([10]).

Thermally processed? Chemically?

Table 1: Proximate composition of raw and processed jack bean seeds

Content	Processed Jack bean	Raw Jack bean
Dry matter (%)	93.10	94.56
Crude protein (%)	28.38	28.90
Crude fat (%)	3.10	2.72
Crude fibre (%)	5.20	7.80
Ash (%)	2.80	3.73
Nitrogen free extract	53.62	51.41

Source: [2]

2. DISCUSSION

2.1 Anti-nutritive constituents of jack bean (*Canavalia ensiformis*).

The use of legumes of livestock nutrition is impeded by the presence anti-nutritional substances in their raw seeds and foliage. Anti-nutritional factors are substances that are found in feedstuff which reduce their nutritional value and also affect the animals adversely in several ways (Table 2). The adverse influence of the dietary raw legumes is associated to the effect of anti-nutritional factors. Alteration of gut permeability, growth retardation, reduction in feed intake, impairment of nutrient digestion and nutrient absorption, hyper-activity of the pancreas, interference and disruptions of cellular metabolic processes, impediment of hormonal and enzyme activities etc. ([5]; [8]; [14]; [31]; [34]; [41]; [42]; [46]; [48]; [52])

The anti-nutritive substances found in jack bean (*Canavalia ensiformis*) seeds are majority: concanavalin A, trypsin inhibitors, the toxic non-protein amino acids (canavanine and canaline), saponins, cyanogenic glycosides and polyphenols. [18]; [3] stated that the occurrence and dietary effect of anti-nutritional factors present in unprocessed plant protein feed ingredients for feeding livestock animals is a significant constraint that decreases nutritional value and their proper utilization.

predominantly

2.2 Concanavalin A

Concanavalin A, a lectin found in jack bean seeds was first described by [57]. Concanavalin A is a potent haemagglutinin and it is capable of agglutinating the red blood cells of several livestock animals and can also react with the sugar components of intestinal cells causing a disruption in

cell structure which consequently produces abnormalities in nutrient absorption. [25] Postulated that lectin-induced disruption of the intestinal cell structure permits invasion of lymph, blood, and liver by bacteria normally confined within the lumen of the gut. Consequently the animal succumbs to otherwise innocuous organisms. Concanavalin A adversely affects nutrient absorption and metabolic processes in the gut. Concanavalin A also reduces intake of feed in non-ruminants ([30]).

Ref. needed.

2.3 Trypsin inhibitors

Trypsin inhibitors are widely distributed within the plant kingdom, including the seeds of most cultivated legumes. They are protease inhibitors occurring in raw legumes seeds and they are the most commonly encountered group of anti-nutritive constituents of plant origin. These anti-nutritional substances inhibit the activity of the proteolytic enzyme (trypsin) in the gut. ([13]; [36]; [38]; [64]). Trypsin inhibitors have been documented to be partially responsible for the growth-retarding property of raw legumes. The growth retardation has been associated with the inhibition of protein digestion but there is evidence of pancreatic hyper-activity, resulting in increased production of trypsin with resultant loss of cysteine and methionine ([38]). Antitrypsin factor found in jack bean seeds are heat-labile ([9]). [6] Confirmed this in their experiment by achieving a total inactivation of antitrypsin activity in jack bean seed and meal after soaking in water for 24 hours before 20 minutes cooking. Trypsin inhibitors are inactivated by heat especially moist heat, because of uniform heat distribution ([11]; [37]).

Toxic non-protein amino acids jack beans seeds: According to [24] the toxic non-protein amino acids jack beans seeds are canavanine and canaline (the product of degradation of canavanine).

2.4 Canavanine

Canavanine is found in most legumes plant *Papilionoideae* ([7]) and abundantly in jack bean (*Canavalia ensiformis*) (L.) (DC.), constituting up to 63g/kg dry weight of seed ([29]). Canavanine toxicity in higher animals is not completely understood ([8]). Canavanine is structurally similar to arginine an essential amino acid, for this reason it antagonizes arginine and impedes its nuclear and cellular metabolic activities, consequently producing a toxic effect. Additionally, its toxicity to insects ([49]) and other organisms ([68]) is well documented and established and the function and role of this non-protein amino acid in plant defence mechanisms was reported by [7]. The deleterious effects of unprocessed *Canavalia ensiformis* for vertebrate animal have, in part, been attributed to the presence of canavanine [17]. [51] posited the interference of the metabolic activity of ribonucleic acid (RNA) by canavanine. This phytochemical may also antagonize arginine.

Research authors ([20]; [39]; [58]) have reported the reduction in feed intake of monogastric animals when the feeding of pure canavanine was done. Notwithstanding, this probably might not be the case when canavanine is present in the seed or meal fed to non-ruminant animals, due to the fact that the release of this factor in the gastro intestinal tract varies when compared to the situation in which canavanine is purely fed to monogastric animals. Canavanine is soluble in water, it is however, also heat stable and this presents a great challenge in terms of the inactivation of this anti-nutritive factor.

2.5 Canaline

Canaline Hydrolytic cleavage of canavanine by arginase results in the production of urea and canaline, a structural analogue of ornithine which is a derivative of arginine. This non-protein amino acid is also found in jack bean seeds. It is also heat-stable, but solubilises in water. The derivative of canavanine, canaline, inhibits the activity of transaminases ([51]).

The structural similarity of canaline to ornithine allows canaline to react with the pyridoxal phosphate moiety of BG-containing enzymes to form a covalently bonded stable Schiff base. Therefore, canaline has been shown to be a potent inhibitor of pyridoxal phosphate enzymes

([47]; [50]). However, canaline toxicity in animals has been suggested to occur in the following ways: by inhibition of enzymes, by competition with ornithine in the arginine urea cycle; or by forming a complex with pyridoxal phosphate cofactor in a manner similar to mimosine ([1]).

2.6 Saponins

Saponins are considered as one of largest classes of natural plant component; however, their phytochemistry and bio-functionality is not completely understood and known in plants ([41]).

Saponins are naturally occurring substances that are vastly distributed in all cells of most legumes. They have the ability to form stable, soap like foams in aqueous solutions, make up a complex and chemically diverse set of compounds ([41]; [54]). Saponins contain a carbohydrate moiety attached to a triterpenoid or steroids. Saponins possess both adverse and beneficial properties [54].

Saponins are known to be temperature sensitive. During processing such as washing, soaking and blanching saponins are dissolved and lost in water [45]. Saponins complexes that are capable of causing damage to the intestinal mucosa and also affects protein digestion in the gut [31] and the absorption of nutrients across the intestinal wall ([14]; [27]). It also known to interfere with the uptake of vitamins and minerals and negatively affects membrane and cell wall permeability ([8]; [26]; [41]).

Saponins have been implicated in the retardation of growth rate, decrease in feed intake, prevention of the activity of smooth muscle ([14]). Saponins have been reported to be detectable in jack beans ([1]; [59]). [59] Found that saponins were not detected after boiling jack beans for the hour.

Generally, saponins and its metabolites can produce several properties that can have either positive or negative effects in different animals ([23]; [56]).

Others: About 1.3% of polyphenols have been reported in jack bean and, like trypsin inhibitors, they are destroyed by moist heat (30 minutes) rather than dry heat ([6]).

2.7 Cyanogenic glycosides

Cyanogenic glycosides are anti-nutritive compounds that are widely distributed in the plant kingdom. Cyanogenic glycosides are products of secondary metabolism. Hydrogen cyanide production is generally dependent upon the cyanogenic glycoside synthesis and the presence or absence of its degradation enzyme. The main toxic substance producing toxicity is hydrogen cyanide (HCN) [66].

Cyanogenic glycosides are majorly vital natural plant components used in the defence mechanism in plant kingdom against herbivores owing to their bitter taste and the release of toxic hydrogen cyanide upon tissue damage or disruption [69].

According to the reports of ([21]) hydrogen cyanide causes the disruption in the metabolic processes of the pentose phosphate pathway, this consequently results in decrease in the rate of glycolysis and inhibiting the tricarboxylic acid cycle. Hydrogen cyanide have also been implicated in the inhibition other vital metabolic activities such as metalloenzymes, many of which contain iron, copper or molybdenum [55].

Jack bean like some other raw legumes contain cyanogenic glycosides from which hydrogen cyanide (HCN) may be released by hydrolysis. Hydrogen cyanide is very toxic at low concentration to animals. It can cause dysfunction of the central nervous system, respiratory failure and cardiac arrest [18]. It has been reported that there is 108mg/kg hydrogen cyanide in raw jack bean seeds [53], although the cyanogenic glycosides and terpenoids in *Canavalia ensiformis* are eliminated within thirty to sixty minutes by boiling (according to the research reports of [59]).

Table 2: Adverse Effects of Anti-nutritive constituents of unprocessed jack bean seeds

Anti-nutritive Compounds	Dietary effects	References
1. Canavanine	- Antagonizes arginine (an essential amino acid) - Impedes nuclear and cellular anabolic activities - Interferes with the metabolic activity of Ribonucleic acid (RNA) - Reduces feed intake	[49] [51] [51] [58]
2. Canaline	- Inhibits the activity of transaminases - Inhibits of pyridoxal phosphate enzymes - Competes with ornithine in the arginine urea cycle.	[51] [47] [50] [1]
3. Saponins	- Causes intestinal mucosa damage - Affects protein digestion in the gut - Affect the absorption of nutrients across the intestinal wall - Causes reduction in growth and feed efficiency - Interferes with the take of vitamins, minerals and adversely affects membrane wall permeability - Prevents the activity of smooth muscle	[31] [31] [27] [14] [26] [14] [26] [8] [41] [14]
4. Cyanogenic Glycoside	- Causes disruption in the metabolic processes of pentose phosphate pathway - Decreases the rate of glycolysis and inhibits the tricarboxylic acid cycle - Inhibits vital metabolic activities of metalloenzymes - It can cause dysfunction of the central nervous system, respiratory failure and cardiac arrest	[21] [21] [55] [18]
5. Concanavalin A	- Capable of agglutinating the red blood cells - Causes disruption in the cellular structure of intestinal mucosa and negatively affects nutrient absorption and metabolic processes in the gut - Reduce intake of feed in non-ruminants	[25] [25] [30]
6. Trypsin Inhibitors	- Produces growth retardation - Pancreatic hyper activity and resultant loss of cysteine and methionine - Causes inhibition of protein digestion - Inhibits the activity of the proteolytic enzyme (trypsin)	[38] [38] [35] [38] [35] [36] [38] [13]

2.8 Dietary effects of jack bean (*Canavalia ensiformis*) seeds on the performance of poultry

The proximate content of jack bean seeds has been reported to be suitable for poultry feeding. ([2]; [28]) reported that the amino acid composition of jack bean protein is very similar to that of roasted soyabean meal with jack bean containing slightly less lysine than soyabean and both having respectively low levels of methionine and cystine.

[25] Fed Japanese quails (*Coturnix coturnix japonica*) with diets containing 500g/kg raw *Canavalia ensiformis* seed and obtained 100% mortality. However, when *Canavalia ensiformis* seeds were autoclaved, no mortality was recorded. [32] Included raw *Canavalia ensiformis* in chick diets at levels of 0, 100, 200 and 300g/kg, mortality increased as the inclusion level increased and reached 50% at the 300g/kg level. In a research study conducted by [15], they reported that autoclaved jack bean seeds included at levels of 100 and 150g/kg in chick diet led to growth rate increases of 76% in comparison with that of the raw seeds. [60] In their experiment showed that dietary inclusion of unprocessed and processed jack bean (*Canavalia ensiformis*) seeds at 20 percent level in the feed, depressed feed intake and percent hen-day egg production with the raw jack bean meal causing greater depression in animal performance.

[28] In their study examined the effect of two lengths of time of autoclaving the seed of jack bean on the performance of chicks fed with the diets at an inclusion level of 300g/kg diet for the whole of an eight-week growing period and for the finishing (weeks five to eight) period only. These workers observed faster growth rates in those birds fed autoclaved *Canavalia ensiformis* for five to eight weeks of age, compared with those that received the same treatment from one to eight weeks of age. This suggests that the older birds were less sensitive to the anti-nutritive effect of *Canavalia ensiformis* than the young birds.

Practical experiment on laying hens and broilers showed a significant decrease in feed intake, feed conversion and growth rate for dietary levels as low 10% raw jack bean seeds ([40]; [67]; [12]).

Research studies carried out by [43] on the effects of raw jack bean meal on performance of broilers, showed that both feed intake and body weight gain were reduced as the dietary level of raw meal increased, but the reduction became significant only at levels above 10% in the diet. According to the research reports of [24], laying birds were observed to be more tolerant, although even in these birds, production of egg was significantly reduced by the inclusion of 200g/jack bean per kilogram of the diet.

Ensiling with ammonia or urea also seems to be successful in decreasing the toxicity of jack beans for chick. The combination of autoclaving and ensiling gave better results than simple ensiling [40]. This is probably because of the solubilisation of the toxic factor in the alkaline (urea) solution [42]. Dry urea ensiling before toasting is effective, it has been shown that up to 200g/kg dietary jack beans treated with dry urea and toasted did not have deleterious effect of broiler performance [63].

[65] Showed that cracking and cooking of jack bean alone is not a good method of processing because dietary inclusion above 20% for broilers resulted in declined performance. Soaking the cracked seeds in urea solution for 24 hours prior to cooking could not appreciably improve its nutritive value when included in broiler diets above 20%. Soaking the cracked jack bean seed in water for 24 hours before cooking for an hour produced the best results, allowing dietary inclusion level of up to 30%. Probably, increasing the period of soaking in water could result in better performance [65].

[22] Reported that detoxified jack bean meal can be included at levels up to 20% in broilers rations without adverse effect on performance and physiological parameters of the broilers.

3. CONCLUSION

Canavalia ensiformis has a protein quality similar to that of most edible food legumes. Jack bean has a great capability as an unconventional plant protein source for livestock feeding however, the thermal-labile and non-thermal-labile antinutritive substances poses some restrictions in its usage for non-ruminant nutrition. Detoxification of the anti-nutritional factors is necessary for the improvement in the nutritional quality of the legume and to effectively utilize their potential as livestock feed ingredients. Another fact that is of interest is that apparently a simple dry cooking process can improve the nutritional quality of jack bean seeds. Thus, the use of the processed seeds in animal feeding, particularly for monogastric animals, is a possibility. Many research authors have reported several processing methods such as; soaking, cooking, toasting, autoclaving, fermentation, extrusion cooking, sprouting, cracking and chemical treatment to improve the quality of the legume, by of the removal or inactivation of anti-nutritional factors. In many instances, for effective and complete detoxification process, a combination of two or more methods may be required. Further research should be geared towards examining the effect of processing methods on the nutritive quality of jack bean and in addition, effort should be intensified on devising ways of improving the palatability of jack bean for poultry feeding, in order to enhance feed intake. Detoxified jack bean (*Canavalia ensiformis*) has high potentials for poultry feeding [22].

REFERENCES

1. Acamovic, T. Analysis and nutritional evaluation for young chicks of some toxic factors in three novel legumes. PhD Thesis. University of Edinburgh. 1987
2. Akande, KE Proximate Composition and Amino Acid Profile of Raw and Processed Seeds of Jack bean (*Canavalia ensiformis*) (L) (DC). African Journal of Natural Sciences. 2004. 7:50-51
3. Akande, KE, Doma, UD, Agu, HO and Adamu, HM. Major anti-nutrients found in plant protein sources: their effect on nutrition. Pakistan Journal of Nutrition. 2010. 9(8): 827-832.
4. Akande, KE Dietary Effects of Increasing Levels of Pigeon Pea Meal on Rabbit Performance. Journal of Agricultural Sciences. 2015. 7(7): 156 -162. doi:10.5539/jas.v7n7p156
5. Aleator, VA and Fetuga, BI. Influence of raw and cooked lima beans on some liver enzyme activity in the rat. Nutrition Reports International. 1984
6. Babar, VS, Chava, JK and Kadam, SS. Effects of heat treatment and germination on trypsin inhibitor activity and polyphenols in jack bean (*Canavalia ensiformis*). Plant Food for Human Nutrition.1988. 38: 319-324.
7. Bell, EA, Lackey, JA and Polhill, RM. Systematic significance of canavanine in the Papilionoideae (Faboideae). Biochemistry of Systems Ecology. 1978 6: 201-212
8. Belmar, R, Nava-Montero, R, Sandoval-Castro C, McNab, JM. Jack bean (*Canavalia ensiformis* (L).DC) in poultry diets: anti-nutritional factors and detoxification studies – a review. World Poultry Science Journal. 1999. 55(1) 37-59
9. Borchers, R and Ackerson, CW. The nutritive value of legume seeds. X. Effects of autoclaving and trypsin inhibitor test for 17 species. Journal of Nutrition. 1950. 41: 339-345.

10. Bressani, R, Brenes, RG, Gracia, A and Elias, LG. Chemical composition, amino acid contents and protein quality of *Canavalia* species seeds. *Journal of the Science of Food and Agriculture*. 1987. 40: 19-23
11. Bressani, R and Sosa, JL. Effects of processing on the nutritive value of *Canavalia* Jack beans (*Canavalia ensiformis* (L.). *Plant Foods for Human Nutrition*, 1990. 40: 207 - 214.
12. Carabano, JM, Armas, A, Madrigal, J and Montilla, J. Posibilidad de incorporar harina de grano de haba de burro (*Canavalia ensiformis*) en raciones para ponedoras. Xxxv Convencion Annual AsoVAC, Merida, Venezuela, p. 96. 1985
13. Carlini, CR and Udedibie ABI. Comparative effects of processing methods on haem agglutinating and antitryptic activities of *Canavalia ensiformis* and *Canavalia braziliensis* seeds. *Journal of Agricultural and Food Chemistry*. 1987. 45 (11): 4372-4377.
14. Cheeke, PR. Nutritional and Physiological implications Saponins: a review. *Canadian Journal of Animal Sciences* 1971. 51: 621-623.
15. Dixon, RM, Escobar, A, Montilla, J Viera, J, Carabano JM, Mora, M, Risso, J, Parra, R and Preston, TR. *Canavalia ensiformis*: A legume for the tropics, In: Recent Advances in Animal Nutrition in Australia (eds. Farrell, D.J. and Vohra, P.) Armidale, University of New England Publishing Unit, pp. 129 – 140. 1983.
16. D'Mello, JPF. Toxic amino acids. In: Anti-nutritional factors, Potentially Toxic Substances in Plants, (eds. JPF D'Mello and CM Duffus). pp. 29-50 (Warwick, Association of Applied Biologists). 1989.
17. D'Mello, JPF, Acamovic, T, and Walker, AG. Nutritive value of jack bean (*Canavalia ensiformis*) for young chicks. *Tropical Agriculture (Trinidad)*. 1985. 62: 145-150.
18. D'Mello, JPF. Anti-nutritional factors and mycotoxins. In: Farm animal metabolism and nutrition. CAB International Wallingford, U.K, pp. 383 - 403. 2000
19. Duke, JA. Handbook of Legumes of World Economic Importance; Plenum Press; New York and London, pp. 39-42. 1981.
20. Enneking, D, Giles, LC, Tate, ME and Davies, RL. L-canavanine: A natural feed-intake inhibitor for pigs (Isolation, identification and significance). *Journal of the Science of Food and Agriculture*, 1993. 61: 315-325.
21. EPA. Summary Review of Health Effects Associated with Hydrogen Cyanide, Health Issue Assessment Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment Office of Research and Development, US Environmental Protection Agency Research Triangle Park, North Carolina, USA. 1990
22. Fabiyi, KE. Utilization of Jack bean (*Canavalia ensiformis* (L).DC) meal as Replacement for Soybean meal in Broiler Diets. M.Sc Thesis, Abubakar Tafawa Balewa University, Bauchi State, Nigeria, 85pp. 1999
23. Francis G, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. *British Journal Nutrition*. 2002 88:587–605.
24. Herrera, F, Gutierrez, M, Cupul, S, Ferriovo, M, Carabano, JM and Montilla, JJ. (1981) The effect of incorporation of *Canavalia ensiformis* seed into a ration for laying hens at 10 and 20%. *Tropical Animal Production*. 1981. 6: 775 – 776

25. Jayne-Williams, DJ. The influence of dietary jack beans (*Canavalia ensiformis*) and concanavalin A on the growth of conventional and gnotobiotic Japanese quail. (*Coturnix coturnix japonica*), Nature, New Biology. 1973. 243: 150-151.
26. Jenkins, KJ and Atwal, AS. Effects of dietary saponins on faecal bile acids and neutral sterols and availability of vitamins A and E in the chick. Journal of Nutritional Biochemistry, 1994. 5: 134 - 137.
27. Johnson, LT, Gee, JM, Price, K, Curl, C and Fenwick, GR. Influence of saponins in gut permeability and active nutrient transport in vitro. Journal of Nutrition, 1986. 116: 2270 - 2272.
28. Kesseler, M, Belmar, R and Ellis, N. Effects of autoclaving on the nutritive value of seed *Canavalia ensiformis* (jack beans) for chicks. Tropical Agriculture (Trinidad). 1990. 67: 116-120.
29. Kitagawa, M and Tomiyama, T. A new amino acid compound in the jack bean and a corresponding new ferment. Journal of Biochemistry (Tokyo), 1929. 11: 265-271.
30. Larue-Achagiotis, C, Pichard, M and Louis-Sylvestre, J. Feeding behaviour in rats on a complete diet containing concanavalin A. Reproduction and Nutrition Development. 1992. 1 32: 34-350.
31. Lásztitya, R, Hidvégia, M and Batab, A. Saponins in food. Food Reviews International, 1998 14 (4): 371-390
32. Laviada, E. Estudios preliminares del efecto de diferentes tratamientos detoxificadores Sobre la semilla *Canavalia ensiformis* y su utilización en la alimentación de aves, M.Sc. Thesis, Universidad Autonoma de ucatan, 1983. Mexico, pp. 24 – 31
33. Leon, AL, Angulo, M, Pichard, B, Carre, L, Deronet JP and Harscoat, JP. Amino acids and starch digestibility and true metabolizable energy content of raw and extruded jack bean (*Canavalia ensiformis*) in adult cockrels. Annale Zootechnology, 1990. 39: 53 – 61
34. Liener, IE. Soyin, a toxic protein from the soybean. 1. Inhibition of rat growth. Journal of Nutrition. 1953. 49: 527-539.
35. Liener, IE. Legume toxins in relation to protein digestibility – a review. Journal of Food Science, 1976. 41: 1076-1081.
36. Liener, IE and Kakade, ML. Protease inhibitors. In: Toxic constituents of plant food stuffs (Editor: I.E. Liener). Academic Press, New York, pp: 7-71. 1980
37. Liener, IE. Possible adverse effects of soyabean anticarcinogens. American Institute of Nutrition, 1995. 125: 7445 - 7505.
38. McDonald, P, Edwards, RA, Greenhalgh, JFD and Morgan, CA. Animal nutrition. Fifth edition. Longman group Ltd, U. K. 607pp. 1995.
39. Michelangeli, C and Vargas, RE. L-canavanine influence feed intake, plasma basic amino acid concentrations and kidney arginase activity in chicks. Journal of Nutrition, 1994. 124: 1081-1087.
40. Montilla, JJ, Ferrero, M, Cupul, S, Gutierrez, M and Preston, T. Preliminary observations: The effects of ensilage and heat treatment on *Canavalia ensiformis* seeds in diets of poultry. Tropical Animal Production. 1981. 6:376-377

41. Moses T, Papadopoulou KK, and Osbourn, K. Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Critical Reviews in Biochemistry and Molecular Biology*. 2014. 49(6): 439–462.
42. Ologhobo, AD, Apata, DF and Oyejide, A. Utilization of raw jack bean (*Canavalia ensiformis*) and jack bean fraction in diets for broiler chicks. *British Poultry Science*. 1993. 34: 323-337.
43. Oyawoye, EO and Ogunkunle, M. Physiological and Biochemical effects of raw jack beans on broilers In: *Proceedings of the Silver Anniversary Conference of the Nigeria Society for Animal Production (NSAP)*. Abeokuta, Nigeria, 21-26. March 1998. pp. 71-72. 1998.
44. Perez, MM. *Canavalia ensiformis* en la alimentacion de pollos de engorda. EQ Thesis, F.M.V.Z – U.A.D.Y. 1984.
45. Price, KR, Johnson, IT, Fenwick, GR and Malinow, MR. The chemistry and biological significance of saponins in foods and feeding-stuffs. *Critical Reviews in Food Science and Nutrition*, 1987. 26(1): 27 - 135.
46. Pusztai, A. Biological effects of dietary lectins. In: *Recent Advances of Research in Anti-nutritional Factors in Legume seeds*; (ed. Huisman) Wageningen, The Netherlands, pp 17-29. 1989.
47. Rahiala, E. Canaline. Characterization of enzyme pyridoxal phosphate complex. *Acta Chemica Scandinavica*. 1973. 27: 3861-3867.
48. Roebuck, BD. Enhancement of pancreatic carcinogenesis of raw soy protein isolate. In: *Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods*, pp. 91 (ed. Friedman, M.). New York, Plenum Press. 1986.
49. Rosenthal, GA. The biological effects and mode of action of L-canavanine, a structural analogue of L-arginine, *Quarterly Review of Biology*, 1977 52: 155-178.
50. Rosenthal, GA. A mechanism of L-Canaline toxicity. *European Journal of Biochemistry* 1981. 114: 301-304.
51. Rosenthal, GA. *Plant Non-Protein Amino and Imino Acids: Biological, Biochemical and toxicological properties* New York and London, Academic Press, pp. 95-113. 1982.
52. Santidrian, S, Reig, G and Miralles, M. (1988). Growth rate, muscle protein composition, intestinal absorption of D-galactose and L-leucine and nitrogen balance in rats fed the raw legume *Chamaecytisus proliferus*. *Plants Foods for Human Nutrition*, 38: 135-143.
53. SECAB. Haba Criolla *Canavalia ensiformis* (L.) DC, (Fabaceae - Fabiodeae). Monografia No. 2. Programa de Recursos vegetales del Convenio Andres Bello (PREVEAB). Secretaria Ejecutiva del, Convenio Andres Bello, SECAB, Bogotá; Colombia, pp. 177-192. 1990
54. Shi, J, Arunasalam, K, Yeung, D, Kakuda, Y, Mittal, G and Jiang, Y. Saponins from edible legumes: chemistry, processing, and health benefits. *J Med Food*. 2004. 7(1):67–78.
55. Solomonson, LP. Cyanide as a metabolic inhibitor. In *Cyanide in Biology*, Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J & Wissing, F. Academic Press, London, New York, Toronto, 11-18. 1981.

56. Sparg, SG, Light, ME, Van Staden, J. Biological activities and distribution of plant saponins. *J Ethnopharmacol.* 2004. 94:219-43
57. Sumner, JB and Howell, ST. Identification of the haem agglutinin of jack bean with concanavalin A. *Journal of Bacteriology*, 1936. 32: 227-237.
58. Tschiersch, B. Über das vorkommen von Canavanin. *Flora*, 1962. 150: 87-94.
59. Udedibie, ABI, Nwaiwu, J, Madubuike F, Nkwocha, CO and Immo, IO. Jack bean (*Canavalia ensiformis*): Poison turned to good protein feed. Federal University of Technology Owerri, Nigeria. 1988.
60. Udedibie, ABI and Madubuike, FN. Effects of dietary raw and cooked jack bean (*Canavalia ensiformis*) on the performance of laying hens. *Journal of Arid Agriculture*, 1988. 1:47-54
61. Udedibie, ABI and Nwaiwu, J. The potential of jack bean (*Canavalia ensiformis*) as animal feed. *Nigerian Agricultural Journal*. 1988. 23: 130-143
62. Udedibie, ABI. Nutritional evaluation of jack bean (*Canavalia ensiformis*) for the Nigerian poultry industry. *Ambio*. 1990 19: 361-365
63. Udedibie, ABI, Esonu, BO, Obaji, CN and Drunna, CS. Dry urea treatment prior to toasting as method of improving the nutritive value of jack bean (*Canavalia ensiformis*) for broilers. *Animal Feed Science and Technology*. 1994. 48: 335-345
64. Udedibie, ABI. Detoxifying Canavalia seeds. *Spore*, 72: 9. 1997.
65. Udedibie, ABI, Esonu, BO and Agbabiaka, LA. Comparative performance of broilers fed diets containing differently processed jack bean meals at finisher phase. *Nigeria Poultry Science Journal*, 2000 1: 32-41
66. Vetter, J. Plant cyanogenic glycosides. *Toxicon* 38(1) :11-36, doi:10.1016/S0041-0101(99)00128-2. 2000
67. Vierma, C. Efecto de varios tratamientos sobre los factores antinutricionales de *Canavalia ensiformis* incluida en dietas para pollos en crecimiento. (Trabajo de ascenso). Facultad de Ciencias Veterinarias U.C.V Maracay, Venezuela. p215. 1984.
68. Walker, JB. Canavanine and homoarginine as anti-metabolites of arginine and lysine in yeast and algae. *Journal of Biological Chemistry*. 1955. 212: 207-215.
69. Zagrobelny, M and Møller, BL. Cyanogenic glucosides in the biological warfare between plants and insects: The Burnet moth-Birdsfoot trefoil model system. *Phytochemistry*, 2011 72(13): 1585-1592, doi:10.1016/j.phytochem.2011.02.023.