THE EFFECT OF TOASTING, DRY UREA TREATMENT AND SPROUTING ON SOME THERMOSTABLE TOXIC FACTORS IN THE JACKBEAN SEED.

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ABSTRACT

Raw unprocessed jackbean seed contains 26 - 32% crude protein and also toxic elements most of which are thermostable, which limit its use as feed ingredient for livestock especially non-ruminant animals. Raw jackbean seeds were divided into three batches. One batch was ground raw and toasted, the second, batch was ground raw and mixed with 2% of its weight of dry urea and allowed to stand for 11 days. The third batch was sprouted for four days and later ground into meal.

Toxicological studies on the three batches of the jackbean meals were conducted for concanavalin A, (Con A), Canatoxin, Urease activity and Haemoglutinin activity. The results of these studies show that dry urea treatment is effective in detoxifying urease activity, Concanavalin A (Con A) and canatoxin in jackbean seed, while sprouting was effective in detoxifying concanavalin A (Con A) and canatoxin but not very effective in detoxifying the urease activity in jackbean seed. Toasting alone did not have appreciable effect on these toxic factors.

Key words:- Jack bean, thermostable toxic factors, toasting, urea treatment, sprouting.

INTRODUCTION

Jackbean has high potentials as energy and protein supplement in livestock feeds. The crude protein content of the ripe seed ranges from 26 to 32% on dry basis and the protein has relatively good amino acid profile. However, jackbean contains a wide variety of chemical substances that are known to exert deleterious effect when ingested by animals especially monogastric animals. The use of these legumes is encumbered by the presence of these proteolytic inhibitors and other anti-nutritional factors, many of which are heat-labile and others heat-stable. Raw jackbean contains saponins, cyanogenic glucocides, terpenoids and traces of alkaloids which cannot be detected after bioling or autoclaving and thermostable anti- nutritional factors such as canavanine and canaline (Rosenthal, 1972), Canatoxin, a non-hemaglutinating toxic protein, (Carlini and Guimaraes, (1981) and more importantly, concanavalin A (Hague, 1975; Jaffe, 1980) which is a lectin. Lectins are reported to affect nutrient utilization by different mechanisms including binding to the glycolipids of the digestive tract mucosa (Hague, 1975; Jaffe, 1980); inhibition of the activity of enzymes of the brush border of enterocytes (Rosenthal, 1972) and interferring with the adherence of enterobacteria to the intestinal wall (Jayne-Williams, 1973).

The nutritive value of jackbean can be higher than that of the groundnut cake once the anti-nutritive factors are removed (Udedibie and Nkwocha, 1990). Urea is a very strong protein- denaturing agent and can achieve this by competing for hydrogen bonds with the peptide backbone thereby breaking up the secondary structure of these native proteins and disrupting their biologically active structures (Rawn-David, 1983).

On the other hand, sprouting initiates three main types of Chemical changes in the seed: the breakdown of certain materials. transport of materials from one part of the seed to another, especially from the endosperm to the embryo or from the cotyledons to the growing parts and finally the synthesis of new materials from the breakdown products formed (Mayer and Poljakoff-Mayber, 1975). The objectives of this study therefore were to determine the

effects of toasting, dry urea treatment and sprouting on some toxic components of jackbean seed.

MATERIALS AND METHODS

EXPERIMENT I:

Raw Jackbean seeds were divided into two batches. One batch was ground into powdery form and then mixed thoroughly with 2% of its weight of urea and stored in a bag. The second batch was ground into a meal raw without mixing with urea. Samples of the urea-treated and raw jackbean meals were analysed for proximate composition (AOAC, 1980) on the day of treatment and for crude protein every other day thereafter. On the 10th day, both the raw and urea-treated jackbean meals were subjected to toasting at temperatures fluctuating between 100 and 120°C until the meals became crispy. The toasting which lasted for 15 - 20 minutes involved adding about one kilogramme of the meal onto a pan already placed on a gas cooker and steadily turning it until it became crispy and dark yellow in colour.

Samples of raw toasted and Urea-treated and toasted jackbean meals were analyzed for concanavalin A (Con A), canatoxin and haemaglutinin activity using gel filtraton in FPLG superose 12 column. Urease activity of the jackbean meals was monitored by determining the crude protein content of the two batches every other day.

EXPERIMENT 2:

Raw jackbean seeds were spread evenly on moist jute bags for 3 - 4 days to facilitate sprouting. Sprouting rate was about 90% on the fourth day. The growth of the sprout was terminated by oven drying at a temperature of 70°C for 24 hours and ground in a hammer mill, using a 2 mm screen. Proximate analysis of samples of the sprouted and ground jackbeans was then conducted using standard methods (AOAC, 1980).

Toxicological studies were conducted as outlined below:- Concanavalin A content was

TABLE 1: PROXIMATE COMPOSITION OF RAW, TOASTED RAW, SPROUTED AND UREA TREATED/TOASTED JACKBEAN MEALS.

	RAW JB	TOASTED RAW JB	SPROUTED JB	UREA TREATED/ TOASTED JB
Crude protein %	28.22	27.92	28.26	26.46
Crude fibre %	7.81	7.66	7.70	7.28
Ether Extract %	3.06	3.04	4.01	3.05
Total Ash %	3.71	3.55	4.05	3.62
Nitrogen Free Extract %	50.2	55.1	45.3	57.58
Calcium	0.14	0.12	0.13	0.10
Phosphorus	0.71	0.63	0.65	0.64
Gross energy (Kcal/g)	4.70	4.68	4.41	4.57

All values expressed on 100% dry matter basis.

TABLE 2: EFFECT OF STORAGE ON CRUDE PROTEIN CONTENT OF RAW AND UREA-TREATED JACKBEAN MEAL

TIME (DAYS)	RAW JB	UREA-TREATED JB
Day 1	25.22	35.21
Day 3	28.26	34.02
Day 5	27.98	29.78
Day 7	28.00	26.47
Day 9	28.14	26.47
Day 11	28.08	26.46

TABLE 3: EFFECT OF RAW TOASTING, SPROUTING AND DRY UREA TREATMENT/TOASTING ON SOME TOXIC FACTORS IN JACKBEAN SEED.

Treatment	mg/ptn seed	Canatoxin u/ g seed	Haemoglutinin activity (mu/g)	Con A mu/g seed	Urease activity mu/g seed
Raw Jackbean	28.22	12.21	8.40	7.23	13.6
Toasted Jackbean	27.92*(3.61)	12.1(0.90)	8.25(.78)	7.00(3.28)	12.8(5.88)
Urea treated/toasted Jackbean	26.46(6.23)	6.11(49.96)	5.04(40.0)	2.89(60.02)	1.5(88.97)
Sprouted Jackbean	28.26(0.0)	1.5(87.71)	4.13(49.0)	4.87(82.76)	9.6(29.4)

*Percent reduction of values is in parenthesis.

measured by hemaglutination of rabbit fresh erythrocytes. Briefly, 25 ul of protein samples were serially (two-fold) diluted in phosphate - buffered saline in 96- microwell plates and 25ul of a 2% v/v erythrocytes suspension was then added to each well. Hemaglutination titers were determined after two hours at room temperature and expressed as minimal amount of protein inducing hemaglutination (Hemaglutination Unit, H.U).

Anti-canatoxin polyclonal antibodies were raised in rabbits as described by Carlini et al, (1988). The immune IgG fraction obtained after at least two boosters were purified according to Carlini *et al* (1988) and further absorbed for canavalin, concanavalin A and Urease.

Canatoxin content was estimated by dot analysis and was done using 0.2m introcellulose membrane as described by Carlini *et al* (1988). After application of 5 ul aliquotes of the serially diluted (5 - fold) protein samples, the membranes were blocked with 5% low fat milk solution and then exposed to rabbit anti- canatoxin IgG polyclonal antibodies (1:10,000). Goat anti-rabbit IgG conjugated to alkaline phosphatase was used as secondary antibodies and color reactions were developed with nitroblue tetrazoluin and 5-bromo-4-chloro-3-indolyl-phosphate in aminomethyl-propanediol, pH 9.6. Results are expressed as the minimal protein concentration producing a colored (+) dot.

Urease activity was estimated by measuring released ammonia colorimetrically using the phenyl-nitro-prussiate-hypochlorite method. One unit of urease activity was defined as the mass of enzyme that will release one micromole of ammonia per minute, at 25°C, in pH 7.0.

RESULTS AND DISCUSSION

The Chemical composition of both the raw, toasted raw, sprouted and urea-treated jackbean meal is shown in Table 1. Urea treated jackbean meal lost about 6.2% of its original crude protein as against 1% by toasted raw jackbean meal. Other nutrients were not seriously affected by the treatments. The action of the Urease of the raw jackbean meal as monitored by nitrogen disappearance from Urea-treated raw jackbean meal with time is shown in Table 2. From the data on the table, it appears that urease activity of the jackbean is rapid, with all the added urea completely hydrolyzed by the 6th day of storage.

The smell of the resultant ammonia gas drastically diminished as from the 6th day. Another important observaton from the data was that by the 6th day of storage the crude protein content of the urea treated jackbean meal dropped below the crude protein of the raw jackbean meal. This indicates that the urease activity was possibly accompanied by some other biochemical degradation which resulted in some nitrogenous compound(s) in the jackbean losing their nitrogen too. Dry urea treatment reduced the concanavalin A level of jackbean by 60%; canatoxin by 50% and heamaglutinin activity by 40%. Toasting alone did not have appreciable effect on these toxic factors (Table 3).

EXPERIMENT 2:

The chemical composition of sprouted ground jackbean meal is shown on Table 1:

and effect of sprouting on the toxic components shown on Table 3.

Sprouting was effective in detoxifying concanavalin A. Recovery of urease, on the other hand was quite expressive in the sprouted jackbean. Results for canatoxin are quite surprising. Sprouted jackbean meal did not show toxicity after i.p injection in mice. However, intraperitoneal toxicity is just one of the multiple effects of canatoxin and most probably not the most reliable one for assessing its effective concentration. Sprouting and urea effectively reduced levels of immunoreactive canatoxin.

The results of these studies have shown that dry urea treatment is effective in detoxifying urease activity, Con A and canatoxin in jackbean seed while sprouting was effective in detoxifying Con A and canatoxin but not very effective in detoxifying the urease activity in jackbean seed. Toasting alone did not have any appreciable effect on these toxic factors.

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