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EFFECTS OF HEATING TEMPERATURE ON THE NUTRITIONAL VALUE OF DELONIX REGIA SEEDS FORWEANER RABBITS FEEDING

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Abstract
The study was carried out to determine the effect of heat treatment on some anti-nutritional factors on the composition of Delonix regia seeds (Hook). The anti-nutritional factors analyzed were Trypsin Inhibitor Activity (TIA), phytate, tannin and cyanide. The seeds were cooked at 100°c for 0 minutes as the control, 15, 30, 45, 60, 75 and 90 minutes respectively. The heat treatment shows that TIA 9.230mg/100g was reduced to 1.81mg/100g after 90 minutes of heating. Phytate 8.27 was reduced to 3.77mg/100g after 90 minutes cooking at 100°c. Tannin 0.43mg/100g was reduced to 0.11mg/100g while cyanide 2.06mg/100g was reduced to 0.57mg/100g after 90 minutes heating at 100°c.Thepercentage of reductions was as follows: 80.59 for TIA, 54.42 for Phytate,74.44 for Tannin and72.33 for Cyanide. Cyanide had the highest reduction in the first 15 minutes 9.79% followed by Tannin 25.38%, Phytate 26.6 and TIA 31.46% in that order. TIA shows the least reduction. The seeds should be cooked for 60 minutes at 100°c to reduce all anti-nutritional factors to a threshold level. The seeds can serve as a good source of protein for rabbit's diets.

Keywords: *Heating Temperature, Anti-Nutritional Factors, Delonix Regia, Seeds.*

Background to the Study

Seeds often contain factors which are deleterious or indeed toxic to animals or man (Liener, 1996). There is a wide distribution of biologically active constituents throughout the plant kingdom, particularly in plants used as animal feed stuff and in human nutrition (Igile, 1996). The knowledge that these compounds elicit both toxic and advantageous biological response has given rise to several investigations in recent times as to their possible physiological implications in various biological systems (Igile, 1996). These anti-nutritional factors need to be removed or inactivated by extensive predetermined heat treatment of seed diet. However, effective pre-treatment is difficult to achieve if there is limited availability of fuel for cooking. This is particularly a serious

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problem in relation to the phytate and other factors since they are quite resistant to heat treatment. Anti-nutritional factors diminish animal productivity but may also cause toxicity during periods of scarcity orconfinement when the feed rich in these substances is consumed by animals in large quantities (Kumar, 1992). The cyanogenic glucose on hydrolysis yields toxic hydrocyanic acid (HCN). The cyanide ion inhibits several enzymes; depress growth through interference with certain nutrients. They also cause acute toxicity, neuropathy and death (Frenando, 1987). Alkaloid cause gastrointestinal and neurological disorder (Aletor, 1993). Tannin cause decreased feed consumption in animals, binds dietary protein and digestive enzymes to form complexes that are not readily digestible (Aletor, 1993). They also cause decreased palatability and reduced growth rate (Roeder, 1995). Trypin Protese inhibitor causes pancreatic enlargement and growth depression (Aletor and Fetuga, 1987). Substances which occur naturally in food manifest their toxicities especially when consumed with food in large or little doses. Some of these dietary or anti-nutritional factors interfere with bioavailability of nutrients and constitute a major factor limiting the wider food use of such tropical plants. The presence of phytate in foods is known to lower the bioavailability of minerals and inhibits several proteolytic enzymes and amylase (Singh et al., 1996).

It is therefore essential that any potential food sources should be examined for anti-nutritional factors. A general survey of anti-nutritional properties of several tropical seeds are been considered for future development is been undertaken on *Delonix regia* seeds.

Objectives of the Study

1. To determine the anti-nutritional factors such as TIA, Phytate acid, Tannin and Cyanide in the seeds of *Delonix regia*.

2. To determine the effect of heat on the anti-nutritional factors of *Delonix regia*.

3. To examine the implications of these anti-nutritional factors on rabbits diets.

Materials and Methods

Anti-Nutritional Factors Analysis

1. Determination of Trypsin Inhibitor Activity (TIA)

Trypsin inhibitor activity of sample was determined by the method of Kakade *et al* (1974). The digest contained 1.0g of the sample, 40g of trypsin and 2.0mg of Benzoiyl (-DL-argine-P-nitroanide (BAPA) in tris buffer. The absorbance of sample was read at 410nm.

2. Determination of Phytic Acid

An indirect calorimetric method of Wheeler and Ferrel (1971) was used for phytate determination. This method depends on an iron to phosphorus ratio of 4:6. Five grams of the test sample was extracted with 3% tri-chloro acetic acid. The phytate was precipitated as ferric phytate and converted sodium hydroxide. The precipitate was dissolved in hot 3.2N HNO and the colour read immediately at 480nm. The standard solution was prepared from Fe $(NO_3)_3$ standard curves. The phytate concentration was calculated from the iron results assuming a 4:6 iron: phosphorus molecular ratio.

3. Determination of Tannin

Tannin contents of samples were determined by the method of Folin-Dennis as described by Pearson (Pearson, 1976).

4. Determination of Cyanide

Cyanogenic glycoside contents of ample were determined by alkaline titration method Wheeler and Ferret (1971). Briefly, samples (1.0g each in triplicate) dissolved in 200ml distilled water were distilled for 2hrs to collect 150cm^3 of distillate. To the distillate, was added 20cm^3 of a 2.5% NaOH and the volume made up to 250cm^3. To samples (100cm^3 of diluted distillate) was added 8.0cm^3 of 6M NH_4OH solution and 2.0cm^3 of 5% KI, and then titrated against 0.02M $AgNO_3$

solution using 10cm^3 micro burette.The end-point was noted as a permanent turbidity against a black background. Titre values were obtained and cyanogenic glycoside contents calculated using the formula.

$$\text{Cyanogenic glycoside mg/100g} = \frac{\text{TV X 1.08 X EV X 100}}{\text{SM X AL}}$$

Where:
TV = Titre Value (cm^3)
EV = Extract Vol. (cm^3)
SM = Sample Mass (g)
AL = Aliqout (cm^3) useds
N/B 1 cm^3 of 0.02N $AgNo_3$ = 1.08mg HCN.

Statistical Analysis:
The data obtained in this work were subjected to statistical analysis using statistical programmes in Microsoft Excel and Statistical Package for the Social Sciences (SPSS 10.0 package). The statistical analysis carried out using mean and standard deviation, analysis of variance (ANOVA). Duncan's Multiple Range (DMR) test (Alder and Roessler, 1977; Ogbeibu, 2005).

Results and Discussion
The effect of heat treatments on some anti-nutritional factors and their percentage reductions in *Delonix regia seeds* when heated at various duration sat 100°C are presented in tables 1 and 2.

Table 1:
Table 1: Anti-nutritional Factors in *Delonix regia* Seeds cooked in 100°c For Varying Time Period

Paremeter	0 min.	15min.	30min.	45min.	60min.	75min.	90min.	SEM	LOS
Trypsin Inhibitor (mg/100g)	9.23^a	6.08^b	3.15^c	2.00^d	1.98^d	1.89^d	1.81^d	0.11^b	*
Phytic Acid (mg/100g)	8.27^c	6.95^b	4.75^c	4.16^d	4.07^{ed}	3.97^e	3.77^e	0.106	*
Tannin (mg/100g)	0.43^a	0.31^b	0.20^c	0.17^c	0.16^c	0.14^c	0.11^d	0.013	*
Cyanide (mg/100g)	2.06	.70^{a}1.56^b	1.29^c	0.64^d	0.60^d	0.57^d	0.0665	*	

Figures followed by the same letter(s) in each row are not significantly different (P<0.05) using DMRT.

SEM: Standard Error of Means

LOS: Level of Significance

*: Significant (P 0<0.05)

NS: Non-significant difference

The values of trypsin inhibitors activity (TIA) ranged from 9.23mg/100 at 0 minute to 1.81mg/100g at 90 minutes heating for 100°C. Trypsin is an enzyme inhibitor (Protase inhibitor) that causes pancreatic enlargement and growth depression. (Aletor and Fetuga 1987) they depress animal growth by interfering with the digestion and absorption of nutrients in the gastrointestinal tract.

Phytate ranged from 8.27mg/100 at 0 minutes to 3.77mg/100 at 90 minutes heating. The percentage reduction ranged from 15.96% to 54.44% for 90 minutes heating at 100°C, Phytates binds minerals like calcium, iron, magnesium and zinc and make them unavailable thus interfering with animal metabolism.

The value of tannin ranged from 0.43mg/100 cooked at 0°c to 0.11mg/100 cooked at 90 minutes. The percentage reduction after heat treatment was in the range of 27.91% at 0°C. to 74.42% at 90 minutes. The reduction in tannin could be attributed to the fact that tannin are polyphenols and all polyphenolic compound are water soluble and can be leached into heat medium (Nworgu *et al.*, 2007).

Tannins are known to inhibit the activities of digestive enzymes and their nutritional effects are mainly related to their interaction with protein. Tannin-protein complexes are insoluble and protein digestibility is decreased by combining with the digestive enzymes of the intestinal tract to form a precipitate. Tannin also reduces the catalytic and hydrolytic functions of enzymes (Murray et al., 2000).

The value of heat ranged from 2.06mg/100g at 0 minutes to 0.57mg/100g after heating for 90 minutes. Heat treatment decreased the level of HCN by 72.33% at 90 minutes. This is similar to that reported by Ajala (2009), Ima-obong and Bassey (2012). Since the release of HCN from glycoside precursor is enzymatic reaction, heat treatment affects the reaction, hence the observed reduction. HCN is known to inhibit cytochrome oxidase, a respiratory enzymes (Murray *et al.*, 2000).

The Results of the Reduction of Anti-Nutritional Factors are presented in Table 2.
The raw *Delonix regia* seeds contain high levels of anti-nutritional factors. There was a significant ($P>0.05$) reduction on the levels of these factors as the duration of cooking increased. The most affected was trypsin inhibitor activity, followed by tannin, phytate and hydrocyanic acid. Boiling for 60 minutes at 100°C was able to reduce trypsin inhibitor, phytate, tannin and hydrocyanic respectively to a threshold level. For these seeds to be used for rabbit's diets, it must be cooked or heated to a temperature (60 minutes at 100°C) that can render the seeds useable for rabbit consumptions. The result clearly demonstrated that trypsin inhibitor had higher susceptibility to heat than other anti-nutritional factors commonly called secondary metabolites. This confirmed an earlier observation by Wanjekechi *et al.*, 2003; Bawa 2003 and Abdullahi and Abdullahi, 2005).

Table 2
Percentage reduction of Antinutritional Factors in *Delonix regia* Seeds

Paremeter	0min.	15min.	30min.	45min.	60min.	75min.	90min.
% Destruction of TIA	0	34.13	65.59	78.33	78.54	79.52	80.39
% Destruction of Phytic acid	0	15.96	42.56	49.70	50.79	52.00	54.44
% Destruction of Tannin	0	27.91	53.49	60.47	62.79	67.44	74.42
% Destruction Cyanide	0	17.48	24.27	37.38	68.93	70.87	72.33

Conclusion and Recommendations

The result of this study indicates that *Delonix regia* seeds can be heated for 60 minutes at 100°C to reduce all anti-nutritional factors to a threshold level.

1. It is therefore, recommended that the seeds should be cooked for 60 minutes at 100°C to reduce all anti-nutritional factors in the seeds.
2. Farmers are encouraged to use *Delonix regia for* ration formulation.
3. The seeds can serve as a good protein when processed at the right temperature.
4. They are palatable if antinutritional factors are reduced.

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