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Effect of Cooking Duration on Chemical Composition and Degradation Characteristics of Rattle Box (*Crotalaria retusa*) Seed using *In Sacco Technique*

Yashim S.M., Tanko M., Yakubu R.L., Abdu S.B., Adamu H.Y and Jokthan G.E.

Department of Animal Science, Ahmadu Bello University, Zaria

*Corresponding author: yashimms@gmail.com

Target Audience: Nutritionist, Animal Scientist

Abstract

Three ruminally fistulated Yankasa rams were used to study the effect of cooking duration on chemical composition and degradation characteristics of rattle box (Crotalaria retusa) seeds using the in sacco degradability method. Crotalaria retusa seeds were subjected to cooking time of 0, 15, 30, and 45 minutes, respectively. Four concentrate diets containing Crotalaria retusa seed meal cooked at 0, 15, 30 and 45 minutes were used for this study at incubation periods 3, 6, 12, 24, 36, and 48 hours. The proximate composition showed that duration of cooking had effect on most of the parameters. The CP contents were 20.60, 21.57, 23.97 and 21.97% for 0, 15, 30, and 45 minutes cooking duration, respectively. All the antinutritional factors analysed showed a decrease with increases in cooking time. Phytate showed a decrease with increase in cooking time from 7.31 to 3.22 at 0 to 45 minutes cooking duration, respectively. The result showed a similar pattern for rate of degradation at 6, 12, 24, 36 and 48 hours incubation time with 45 minutes cooking duration significantly (P<0.05) recorded the highest values of 47.00, 54.67, 73.00, 87.00, 90.67 and 93.00 percent disappearance of the experimental feed, respectively. The highest degradability (a+b) was in 0 minutes cooking duration, which was significantly (P<0.05) higher than in 15 and 30 minutes cooking. The rate of degradation constant (c) was significantly (P<0.05) lower in 0, 15 and 30 minutes cooking duration as compared to 45 minutes cooking duration. Effective dry matter degradation significantly (P<0.05) decreased with cooking duration in out flow rate (k). It can be concluded from this study that, cooking duration affects degradation characteristics of C. retusa seeds.

Keywords: *Crotalaria retusa* seed, cooking duration, in sacco, rumen degradation

Description of Problem

Ruminant diets in most developing countries are based on roughages and crop residues. These feeds are inbalanced and are particularly deficient in protein, minerals, vitamins, and are highly lignified. Efficient supplementation of locally mixed concentrate with grains or protein foliages has been demonstrated to improve rumen ecology, dry matter intake, and subsequently meat and milk quantity and quality (1). Tree leaves and seeds have high protein contents (18 - 26%) and some of them have low rate of

degradability in the rumen (2). These characteristics, along with those mentioned above, make them an alternative of by-pass protein to be used as a supplement for ruminant production systems in the tropics. Legume is a broad word that refers to plants like shrubs, forbs or trees. Legumes are plants that have a reasonable quantity of crude protein content and are also rich in minerals required for proper growth, pregnancy, and lactation. These plants are nitrogen fixers with the aid of bacteria in the root nodules of the plants.

Maximizing the use of legume forage in feeding small ruminants and selection for varieties that are dual purpose to supply quality protein requirement for the ruminant animals as the conventional legume crops have been advocated (3,4).

A major constraint to the use of legumes and their seeds as livestock feed is the presence of toxic antinutritional constituents. These constituents have different but adverse effects on animal performance including loss of appetite and reduction in dry matter intake and protein digestibility. *Tannins* inhibit the utilization of nutrients through enzyme inhibition and reduced forage digestibility (5). *Phytate* interfere with the absorption and utilization of several mineral elements especially Ca, Mg, Fe and Mo. *Oxalates* affect Ca and Mg metabolism (6). The *saponins* act on the cardiovascular and nervous systems as well as on the digestive system. Large doses of legume juices containing saponins cause distention of the rumen (7).

There is limited information on the nutritive value of *Crotalaria retusa* (Rattle box) seed. Like any other tropical legumes, its usefulness as animal feed ingredient may be limited due to presence of some antinutritional factors (8). It has been established that boiling, soaking, germination, genetic engineering and antioxidants present in some grain legume exert beneficial effect by destroying the antinutritional factors inherent in legume grains (9).

The present study was conducted to evaluate effect of duration of cooking of *Crotalaria retusa* seeds supplemented with concentrate diets in fistulated Yankasa rams.

Materials and Methods

Seed processing

The experimental materials rattlebox seeds (RBS) were collected from the Institute for Agricultural Research Farm, Ahmadu Bello University, Zaria. The seeds were later threshed after drying. The processing of the seeds was done in batches of 1kg *Crotalaria retusa* seeds were subjected to four cooking durations of 0, 15, 30, and 45 minutes. Each of the cooking duration represented a treatment. For each cooking duration, 4 litres of water was first brought to boiling point in a 10 litre pot. A batch of 1kg *C. retusa* seed were then poured into the boiling water, from this point, the specified duration for cooking was taken. At the end of the period of cooking, excess

water was drained off. This was repeated for all the cooking durations. The cooked seeds were then spread thinly on a polythene material on a concrete floor and left to dry for 5 days with occasional stirring. The cooked seeds were milled and then samples taken for Laboratory analysis.

Proximate analysis

The dry matter content was determined based on the weight loss after 24 hours in an oven at 100^{o}C. Nitrogen content was determined by the Macro Kjeldahl Method of (10) and the crude protein calculated as N x 6.25. The ash content was determined as the residue remaining after incinerating the sample at 600^{o}C for 3 hours in a muffle furnace. The (10) methods were employed for ether extracts, crude fibre and all other proximate components determinations. The antinutritional factors hydrogen cyanide, phytate, trypsin inhibitor and saponin were determined using the procedure (10) at National Research Institute for Chemical Technology (NARICT), Zaria.

Experimental Animals and their management

Three fistulated Yankasa sheep with an average weight 28kg were used for this study. The animals were placed in individual pens and fed maize stover *ad libitum* as basal diet. Each ram was fed with concentrate supplementary diet of 1.5 % of its body weight per day. The animals were given free access to mineral-licks and clean drinking water for the duration of the experiment. The animals were adapted to basal feed for two weeks prior to suspension of the nylon bags.

Supplementary concentrates diets

Four supplementary concentrate diets with 0, 15, 30 and 45 minutes cooking duration of *Crotalaria retusa* seed meal were formulated to contain 12% crude protein (Table 1). Other ingredients used for the supplementary diets include maize, cottonseed cake, wheat offal, rice offal, salt and bone meal.

Rumen degradability measurement

The nylon bag technique as described by (11) was used to determine the dry matter disappearance of concentrate containing *C. retusa* seed cooked at different duration of 0, 15, 30, and 45 minutes. Nylon bags (90 mm x 120 mm) of known weights with approximate pore size of 40μ were used. Three grammes (3g) of each sample per treatment were weighed in triplicates into nylon bags which were firmly fitted into slit plastic tubes, labelled according to the incubation hour and inserted into the rumen of the rams. Thereafter the nylon bags were removed at 3, 6, 12, 24, 36, and 48h and immediately kept inside a flask containing ice to terminate fermentation. Zero hour washing loss was determined by soaking 3 replicates of each sample in warm water for 30 minutes. All the bags were then rinsed under tap water until the rinsed water became clear, and then placed on metal trays. These were later oven-dried at 60^{o}C for 48 h, weighed to determine the dry matter loss. Determination of degradation

characteristic Constants, and dry matter disappearance were fitted into the exponential equation:

$P=a + b (1-e^{-ct})$ as reported by (11)

where P= dry matter degradation at time t
a= soluble water fraction
b= insoluble fraction that will degrade in time t
c= degradation rate constant (% h^{-1}) of the b fraction.

The constants were used in computing the potential degradation (PD) and effective degradation (ED), as

PD = Potential degradability or extent of degradation (a + b) in time t
ED was estimated from the equation (11).

$ED= a + (bc/c+k)$

Where k is the rumen outflow rate of digesta at 0.02, 0.03, 0.04 and 0.05 h^{-1}

Statistical analysis

Data generated from the degradation studies were subjected to analysis of Variance using Statistical Analysis Systems (12) in a completely randomized design. Significant differences between treatment means were compared using Duncan's Multiple Range Tests.

Table 1: Composition of the experimental diets

	Duration of cooking *C. retusa* meal (mins)			
Constituents	0	15	30	45
Maize	32	27	23	18
Cotton seed cake	36	26	15	5
Rattle box seed	-	15	30	45
Wheat bran	20	20	20	20
Rice bran	10	10	10	10
Bone meal	1.5	1.5	1.5	1.5
Common salt	0.50	0.50	0.50	0.50
Total	100	100	100	100

Results and Discussion

The results of proximate composition of cooked rattle-box seeds are presented in Table 2. The results shows that duration of cooking had effect on most of the parameters. The values of crude protein (CP) were not consistent with the increase in cooking duration. Cooking at 30 minutes gave a higher 23.97% (CP) than compared to cooking at 0, 15 and 45 minutes, which produced 20.60%, 21.57% and 21.97% of crude protein, respectively. These values compared favourably with the values obtained from whole cotton seed (20.0%) and sesame seed (24.2%), respectively (13).

The values of ether extract (EE) for 45 minutes cooking time were higher than other cooking durations followed by 15

minutes cooking time. The EE values were generally lower than that of ground nut cake (9.16%) but compare favourably with those of sesame seed and palm kernel which were 7.0% as reported by (13), indicating that RBS may not be an oil seed.

Table 2. Effect of cooking duration on proximate composition of *Crotalaria retusa* seeds.

	Duration of Cooking (mins)			
Component (%)	0	15	30	45
Dry Matter	93.27	94.09	93.81	95.08
Crude protein	20.60	21.57	23.97	21.97
Crude fibre	15.74	15.38	15.53	15.37
Oil	7.72	8.27	6.10	8.61
Ash	6.28	6.57	5.29	6.50
Nitrogen free extract	49.67	48.23	49.12	47.56

Table 3 shows the effect of duration of cooking on antinutritional factors of *C. retusa* seed meal. All the ANFs analysed decreased with increases in cooking time. Phytate content decreased with increase in cooking duration from 7.31 to 3.22 at 0 to 45 minutes, respectively. The reduction in the level of phytate was in agreement with the findings of (14), who observed that decrease in phytic acid content of legume seeds during cooking may be partly due to formation of insoluble complexes between phytate and other components.

Hydrocyanide (HCN) content also decreased with increase in cooking duration from 0 to 45 minutes. This decreasing pattern of HCN is in agreement with the findings of (14) who reported a decreased in HCN with increased in cooking duration. Free and bond cyanide are both soluble and might have leached out during cooking or vaporizes into the atmosphere. Trypsin inhibitor activity decreased with increase in cooking time as observed in other antinutritional factors. This was in line with the findings of (15) who observed a linear decrease of trypsin inhibitor with increase in cooking duration in Chicken pea *(Cicer arietinum L.)*. The results obtained in ANFs is similar to the findings of (9) who observed that most ANFs in legume seeds are destroyed after cooking the seeds for a minimum of 30 minutes.

Table 3. Effect of duration of cooking on the Antinutritional factors of Rattle box seeds.

	Duration of Cooking (mins)			
Factors	0	15	30	45
Phytate	7.31	7.29	4.31	3.22
Saponin	15.26	8.35	7.35	7.25
Hydrocyanide	2.36	2.19	1.84	1.49
Trypsin inhibitor	73.30	65.00	49.80	36.45

Table 4 shows disappearance of experimental material after incubation in the rumen of rams. Ruminal degradation of the material increased with increase in incubation time from 0 to 48 hours for all treatments. There was significant ($P< 0.05$) difference in the degradation following different processing techniques. T_1 had the lowest degradation of 41.00% for 3 hours incubation time. However, there was no significant ($P>0.05$) differences between T_2 (47.68%), T_3 (47.67%) and T_4 (47.00%). The result showed a similar pattern of rate of digestibility for both degradation at 6, 12, 24, 36 and 48 hours incubation time with 45 minutes cooking duration producing the highest values, which could be due to the differences in chemical composition at different cooking time. This finding is in agreement with (16, 17), who observed that different rates of digestibility in the rumen could be attributed to the chemical composition of the feed. Incubation at 48 hours had the highest degradation value of 93.00%, which can be attributed to the reduction of the ANFs present in the seed meal (9).

Table 4. Percentage Disappearance of Experimental Material after Incubation

	Incubation time (hrs)					
Duration of cooking (mins)	3	6	12	24	36	48
0	41.00^{b}	60.33^{a}	66.67^{ab}	81.33^{b}	88.00^{b}	91.00^{c}
15	47.68^{a}	54.00^{b}	62.67^{b}	85.67^{ab}	89.00^{ab}	92.00^{b}
30	47.67^{a}	54.00^{b}	67.00^{ab}	88.67^{a}	89.67^{ab}	90.67^{c}
45	47.00^{a}	54.67^{a}	73.00^{a}	87.00^{a}	90.67^{a}	93.00^{a}
LOS	*	*	*	*	*	*
SEM	0.57	0.63	0.54	0.44	0.31	0.37

a,b,c Means within column with different superscript are significantly different
LOS = Level of Significance SEM= Standard Error of Means * = $P<0.05$

The effect of processing on degradation characteristics of *Crotalaria retusa* seed is shown in Table 5. The 'a' value, (soluble fraction) obtained for T_4 (45 minutes cooking duration) was significantly ($p<0.05$) lower than that of T_1 (43.00%) and T_2 (38.00%), which was not significantly ($p<0.05$) different with T_3 (33.8%). This could be due to differences in fibre content of the seeds as reported by (18) that the degree of lignifications has a negative effect on cell wall digestibility in forage.

The 'b' value (insoluble but degradable fraction) shows no significant ($P>0.05$) difference between the treatments. Treatment four (T_4) had the highest value of 93.80%, while T_1 had the lowest value of 91.00%. The difference could be as a result of the cooking effect of the seed.

The asymptote (a+b) values differed significantly ($P<0.05$) across the four treatments with T_1 having the highest value of (134.00%), and T_3 had the lowest (126.2%). There was no significant difference between T_2 (130.00%) and T_2 (127.60 %,). The difference observed in the degradation of the treatment could be due to difference in their tannin contents which might have reduced cell wall digestibility (19).

The rate of degradation constant 'c' is an important parameter in the assessment of the fermentation in the rumen. In this study the rate of degradation constant was significantly ($P<0.05$) affected by cooking duration of the seed, with the highest value observed in treatment T_4 (0.629%), while T_3 had the lowest value (0.528%). The T_3 had rate of degradation of water insoluble fraction (c) suggests that T_4 degrades faster than T_1, T_2 and T_3 respectively, this could be as a result of reduction in antinutritive factors due to cooking (20, 21).

Table 6 shows the effect of cooking duration on dry matter degradability. There was significant ($P<0.05$) difference between the four treatments, where K is the rate of particulate outflow from the rumen, it ranges from 0.02 to 0.05 by (11),

Table 5. Effect of cooking duration on ruminal DM disappearance and Degradation Characteristics of experimental diets incubated in the rumen

	Degradation Characteristics			
Treatments	a	b	a+b	C
(T_1 0 Minute boiling)	43.0[a]	91.00	134.00[a]	0.53[b]
(T_2 15 Minute boiling)	38.0[b]	92.00	130.00[b]	0.53[b]
(T_3 30 Minute boiling)	34.0[c]	92.27	126.67[c]	0.53[b]
(T_4 45 Minute boiling)	33.8[c]	93.80	127.60[b]	0.63[a]
LOS	*	NS	*	*
SEM	-	0.377	0.377	0.061

a,b,c Means within column with different superscript are significantly different LOS = Level of Significance SEM= Standard Error of Means *= P<0.05, NS = Not Significant

Where a= is the soluble fraction

b= Insoluble but degradable fraction

a+b= asymptote

c= rate of degradation

Table 6. Effect dry matter Degradability of experimental diets at different passage rate

	Duration of Cooking (mins)				
Rate of passage (K)	0	15	30	45	SEM
0.02	130.63[a]	126.57[b]	121.30[c]	127.10[b]	0.344
0.03	129.10[a]	125.00[b]	119.73[c]	125.70[b]	0.339
0.04	127.60[a]	123.47[b]	118.23[c]	125.26[b]	0.301
0.05	126.10[a]	121.97[b]	116.77[c]	123.10[ab]	0.113

a,b,c Means within row with different superscript are significantly different LOS = Level of Significance SEM= Standard Error of Means * P<0.05

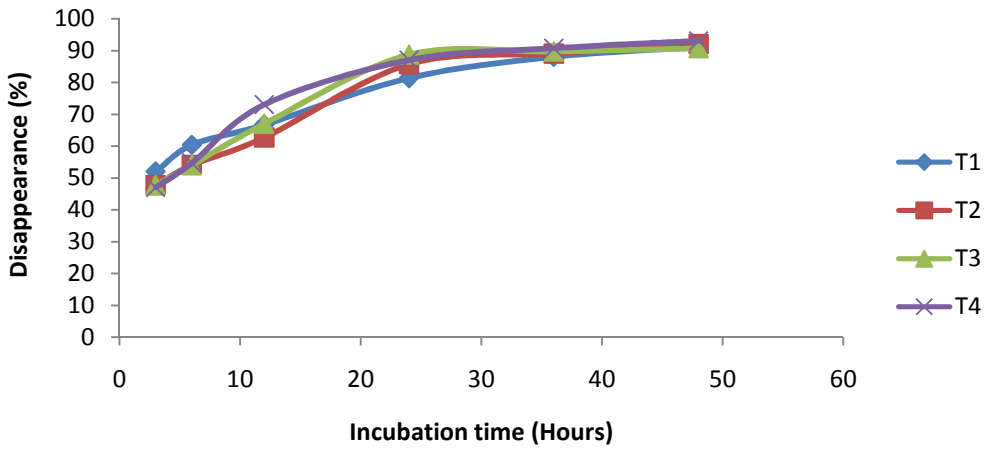

Fig 1. Degradation characteristics of rattle box seed

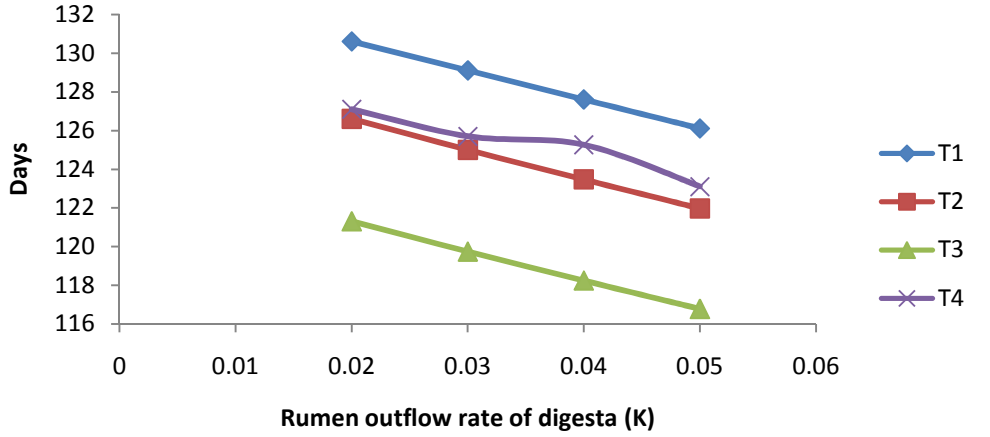

Fig 2. Dry Matter Degradability Graph

Conclusion and applications

1. The result from this study confirmed that cooking of legume seed as a processing method reduced antinutritional factors, thereby improving the nutrient content.
2. It can be concluded that Rattle box seed (*C. retusa*) had a high nutrient profile and potential for feeding animals.
3. The degradability characteristics of *C. retusa* seed is affected by cooking duration.

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