NUTRITIONAL EVALUATION OF RUBBER (*Hevea brasiliensis*) SEED CAKE AS A POTENTIAL LIVESTOCK FEED RESOURCE

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ABSTRACT

Rubber Seed Cake (RSC) was subjected to chemical analysis of proximate, gross energy, minerals, carotenoid and anti-nutritive factors. The analysis revealed that RSC contained dry matter, 90.77±0.06%; crude protein, 14.37±0.15%; crude fibre, 3.70±0.10%; ash, 4.43±0.12%; ether extract, 16.87±0.15% and gross energy (2,141.01±6.0 Kcal/kg). Values obtained (g/100gDM) for calcium (181.7±3.0), iron (8.27±0.15), potassium (34.10±0.9), magnesium (34.17±1.44), manganese (0.047±0.01), sodium (286.7±2.9), lead (0.01±0.001), zinc (0.567±0.06), selenium (0.01±0.001), phosphate ions (125±1.00) and sulphate ions (23.0±1.00) were within the range of values earlier reported for RSC in the literature. Lead level obtained is below the deleterious level. Qualitative and quantitative analyses of RSC revealed that it contained (mg/100g) saponins (160.0); tannins (12.0); oxalates (25.0); phenols (16.83); steroids (86.67); flavonoids (125.0) and phytates (45.0). The values obtained for these anti-nutritional factors are within tolerable levels and of no deleterious effect on animals if RSC is used in formulating their feeds. Carotenoid, terpenoids and cyanogenetic glycosides were however not detected in RSC studied. Based on its chemical composition, the Nigerian RSC is therefore a promising and potentially cheap feed ingredient for use in livestock production by local and large scale farmers as it can replace or substitute energy furnishing ingredients and other ingredients without imparting any harmful effect on the animals and their products.

Key words: Rubber seed cake, Proximate composition, Minerals, Anti-nutritive factors, Feed ingredients.

J. Agric. Prod. & Tech.2013; 2(2):51-60

INTRODUCTION

It had been overstressed over time that the ever increasing scarcity and skyrocketing costs of conventional feed ingredients being imported or produced locally in Nigeria warrants urgent substitution or replacement by locally produced and sub-harnessed alternative feed ingredients in livestock production. The need for alternatives is to reduce the cost of production, increase the profit margin, make livestock farming more attractive to farmers and unemployed youths and invariably make food protein more available on the table. Nwokolo and Akpakunam, (1986) postulated that if the livestock sub-sector of Nigerian economy will grow, it must be based squarely on local, available and potentially available feed ingredients. One of the ways of achieving this is through the evaluation of Rubber Seed Cake (RSC) for use in livestock production as it could substitute the scarce and expensive conventional feed ingredients like soyabean meal and groundnut cake in livestock diets. The RSC is the residue or product of conditioned, milled and de-oiled rubber seed. Rubber seed cake is cheap and available at a lower cost as it is not consumed or used directly by humans. Rubber seed is the round or globoid shaped, brownish and hard substance that is released when the mature and dried rubber fruit capsule dehisce by explosive mechanism usually as from late August to October of the year.

Nigeria has 247,100 hectares of land under rubber cultivation and majority of these hectares are owned by small-scale farmers (Aigbekaen *et al*; 2000; Delabarre and Sevier, 2000) mainly in the southern states of Ogun, Ondo, Edo, Delta, Abia, Rivers, Akwa Ibom and Cross river states. Large quantities of rubber seeds are produced by rubber trees in Nigeria and only a negligible quantity are used to produce rubber seed oil in Rubber Research Institute of Nigeria (RRIN) mainly for research while its cake is either thrown away or used as manure. About 99.9% of the seeds are allowed to waste, rot away in the field or germinate as weeds under rubber plantation thereby making the farmer or the country to lose a lot of money that could have accrued from rubber seed oil and cake if these seeds were processed into useful products besides the provision to the unemployment.

The need to evaluate or re-validate the nutritional composition of rubber seed cake in a bid to suggest, harness or prescribe and encourage its use in livestock production in Nigeria is a cause for this study.

MATERIALS AND METHODS

Source and Procurement of Rubber seed cake: Rubber seed cake used in this study was collected from different batches of RSC produced from Rubber seed processing unit of Rubber Research Institute of Nigeria (RRIN), Iyanomo, Benin City, Edo State, Nigeria.

Chemical Analysis: The RSC was subjected to chemical analysis. The Dry Matter (DM) contents were determined by oven-drying samples at 105^0C to a constant weight. The Crude Protein (CP), Crude Fibre (CF), Ether extract (EE) and ash were determined according to the procedure of AOAC (2010). Nitrogen Free Extract (NFE) and carbohydrate content were estimated from the values obtained. That is NFE = 100 – (CP + Ash + EE +CF) while the carbohydrate content is the sum of the NFE and the crude fibre content. The gross energy values were calculated from the relationship that exists between fat, crude protein and carbohydrate (i.e. 39.4EE + 23.5CP + 17.7 carbohydrates) according to Fisher (1982).

The mineral contents (elements) of RSC in terms of Mg, Mn, Na, Pb, Zn and Fe were determined using the atomic spectrophotometer as described by the methods of AOAC (2010). Phosphorus and calcium were determined calorimetrically (AOAC, 2010). Carotenids were determined using HPLC methodology (AOAC, 2010). Each sample was analysed in triplicates and all readings were done in duplicates.

Qualitative test for anti-nutritional factors: The procedures of Sofowora, (1993); Marcano and Hasenawa, (1991); Treatise and Evans (1989) and Harborne, (1973) were used for the analysis. Dried RSC was milled to powder.

Test for saponins: To 10g of the powdered sample was added 95% ethanol and boiled for about 3 minutes and filtered. 5ml of distilled water was added to the filtrate and shaken vigorously for 1 minute and allowed to stand for 30 minutes. Honey comb-like frothing would indicate the presence of saponins (Sofowora, 1993).

Test for tannins: A 10% ferric chloride solution was added to a part of the filtrate and observed for any formation of precipitates and any colour change. A blue or green colour precipitate would indicate the presence of tannins (Treatise and Evans, 1989).

Test for oxalates: Calcium chloride was added to aqueous solution of the sample. A white precipitate (of calcium oxalate) which is insoluble in acetic acid indicates that oxalate ion is present. Alternatively, addition of acidified $KMNO_4$ is added to the aqueous solution of the sample. The solution will turn colourless if oxalate ion is present. Violet manganate ions would be decolourized into colourless manganous ions with the evolution of CO_2 gas that turns lime water milky (WikiBooks, 2013).

Test for phenols: Five (5) grams of powdered sample was weighed and 95% methanol was added and allowed to stand for 3 days after which the content was filtered with Whatman filter paper (No. 1). Equal volumes of the extract and $FeCl_2$ were shaken together. Deep bluish-green solution was formed with phenols (Sofowora, 1993; Treatise and Evans, 1989).

Test for steroids: 2ml of acetic anhydride was added to 0.5g of ethanolic extract of each sample with 2ml of tetraoxosulphste (IV) acid. The colour change was noticed. If it changes from violet to blue or green, it indicates the presence of steroid according to Akinyeye and Olatunya, (2014); Sofowora, (1993).

Test for flavonoids: To 5 ml of dilute ammonia solution was added to a portion in the aqueous filtrate of the sample extract followed by addition of concentrated H_2SO_4, a yellow colouration will indicate the presence of flavonoids (Sofowora, 1993).

Test for terpenoids: To 5ml of the aqueous extract was mixed with 2ml of chloroform in a test tube and 3ml of concentrated H_2SO_4 was carefully added slowly by the side to the mixture to form a layer. A reddish-brown interface formed indicated the presence of terpenoids.

Test for cyanogenetic glycosides (mg/100g): To 5ml of the extract was added 2.5ml of dilute H_2SO_4 in a test tube and boiled for 15minotes, cooled and neutralized with 10% NaOH. Fehling's solution A and B were added. A brick red precipitation of reducing sugars would indicate the presence of glycosides.

Test for carotenoids: 1g of milled RSC was extracted with 10ml of chloroform in a test tube with vigorous shaking and the mixture was filtered on addition of 85% H_2SO_4, Carotenoids is present if there is a blue colour at the interface.

Determination of saponin content: 1g of the sample was weighed and 5ml of 20% ethanol was added and put in a water bath at 55 ^{0}C for 4 hours. It was filtered and the residue washed with 20% ethanol twice. The extract was reduced to about 5ml in the oven and 5ml of petroleum ether was added to the concentrated extract inside a separating funnel. The petroleum ether layer was discarded and 3ml of butanol was added to it and washed with 5% sodium chloride. The butanol was poured into a weighed petri dish and put in the oven to evaporate to dryness. The residue was then weighed and saponins content calculated as:

$$\% \text{ Saponin} = \frac{\text{Weight of saponin} \times 100}{\text{Weight of sample}}$$

Determination of tannin content: 1g of the sample was extracted with 25ml of the solvent mixture of 80:20 acetone: 10% glacial acetic acid for 5hours. It was filtered and the absorbance measured at 500nm. The absorbance of the reagent blank was also measured. A standard graph was made with 10, 20, 30, 40 and 50mg/100g of tannic acid. The concentrated of tannin was read off taking into consideration the dilution factor.

Determination of oxalate content: 1g of the sample was weighed into 100ml conical flask. 75ml of 15M tetraoxosulphate (VI) acid was added and the solution was carefully stirred intermittently with a magnetic stirrer for about 1hour and then filtered using Whatman filter paper No. 42 (125 mm). 25ml of the sample filtrate (extract) was collected and titrated hot (80 – 90^{o}C) against 0.1M $KMnO_4$ solution to the point when a faint pink colour appeared that persisted for at least 30seconds (Akinyeye and Olatunya, 2014)

Determination of phenols content: It was determined by spectrophotometer using Folin-Ciolcalteau reagent (AOAC., 2010). Two (2) grammes of the sample was extracted with 20ml of a mixture (80:20) of acetone and 0.5% formic acid for 2 minutes and was filtered. 2ml of the extract was then mixed with 0.5 ml of Folin-Ciocalteau reagent and 1.5ml sodium carbonate (20%). It was mixed for 15 seconds and allowed to stand at 40^{0}C for 30 minutes to develop colour. The absorbance on spectrophotometer at 765nm was read and measured; and the phenol concentration is obtained from a standard graph.

Determination of steroid content: 5g of the sample is measured and 100ml of water was added. 0.1m ammonium hydroxide was added to take the pH to 9.1. Thereafter, 2ml petroleum ether, 3ml acetic acid anhydride and concentrated H_2SO_4 were added. The absorbance was read at 420nm.

Determination of flavonoid content: 5g of the sample was boiled in 100ml of 2M HCl solution under reflux for 40 minutes. It was allowed to cool before being filtered. The filtrate was treated with equal volume of ethyl acetate and the mixture was transferred to a separation funnel. The flavonoids extract is contained in the ethyl acetate portion is received by filtration using weighed filter paper. It was oven dried and cooled in desiccators and weighed (Harborne, 1973). The weight was expressed as a percentage of the weight analysed and calculated as:

$$\%\text{Flavonoids} = \frac{W_2 - W_1 \times 100}{\text{Wt. of sample}}$$

W_1 = Weight of filter paper and flavonoid precipitate.
W_2 = Weight of filter paper alone.

Determination of phytate content: 1g of the sample was extracted with 0.2M HCl. To 0.5ml of extract was added 1ml Fe^{3+} solution (ferric ammonium sulphate). To 1ml of supernatant was added 1.5ml of 2, 2-Bipyridine solution. The absorbance was measured at 519nm with distilled water as blank.

Statistical Analysis: Data obtained were subjected to descriptive analysis of GENSTAT (2005).

RESULTS AND DISCUSSION

The proximate and gross energy composition of rubber seed cake is as presented in table 1. The analysis revealed that Rubber Seed Cake (RSC) contained dry matter, 90.77%; crude protein, 14.37%; crude fibre, 3.70%; ash, 4.43% and ether extract, 16.87%.

The dry matter content obtained for RSC in this study was within the range of values (88.9 – 91.90%) earlier reported (Heuse and Tran, 2011; Sovanno, 2002; Hao and Liem, 2003; Narahari et al., 1984).

The Crude Protein (CP) content obtained is within the range of values (12.10 – 18.90%) reported by Narahari *et al.* (1984); Jayasuriya *et al.* (1982); Heuse and Tran (2011) but lower than 29.4 – 30.0% reported by Hao and Liem, (2003). The lower CP can be attributed to the presence of the shell or lignin in the cake that decreased the proportion of nitrogen in the sample. In contrast, Tean *et al.* (2002) however reported a very low value of 8.31% CP for Cambodia RSC.

The ash content (4.43%) obtained is also within the range of values (2.7 – 7.0%) earlier reported (Heuze and Tran, 2011; Hao and Liem, 2003; Tean *et al.*, 2002; Sovanno, 2002). The ether extract content of RSC obtained in this study (16.87%) is higher than the values (2.2 -9.9%) reported by Heuze and Tran, (2011), Tean *et al.*, (2002), Narahari *et al.*, (1984). Sovanno, (2002) had reported 14.7% ether extract for extracted rubber seed kernels in Cambodia. Variation in the fat content of RSC could depend on the method and extent of oil extraction from the rubber seed and the varieties or clones of rubber trees where the seed is obtained. There are many rubber clones in RRIN which includes those developed in RRIN like NIG 800, NIG 801, NIG 803, RRIN 600, NIG 901 – NIG 910 and exotic clones like GT1, PR 107, RRIC 45, PB 219, *GT 1, PR 107 and PB 5/51,* RRIC 45, RRIM 600, RRIM 614, PB 217, IAN 710 and PR 107. Rubber seed had been reported to be rich in highly unsaturated vegetable oil (Sonanno, 2002) and fatty acids like myristic, palmitic, stearic, oleic, linoleic and linolenic acids that are comparable with those of melon seed, palm kernel, coconut and corn oils (Nwokolo and Akpakunam, 1986).

The crude fibre content of RSC (3.70%) is lower than 23.7 – 46.6% reported by Heuze and Tran, (2011) and 6.6% reported by Sovanno, (2002) for Cambodia RSC. Lower values for crude fibre could be attributed to the higher proportion of ether extract in the cake and method of processing that involved drying at 60 – 70% for 12 hours and conditioning for 10 – 20 minutes at 60 – 70^0C (Iyayi *et al.*, 2008).

Based on the proximate composition of RSC, carbohydrate and gross energy composition obtained in this study, rubber seed cake compares favourably with conventional feed ingredients like wheat offal, palm kernel cake, Brewer Dry Grain (BDG), rice bran in protein, fatty acids and energy composition; and can replace up to 100% of these ingredients in livestock ration. It can also replace substantial proportion of protein and energy furnishing ingredients like ground nut cake, soybean meal, maize offal and other cereals in livestock feed. It would be a valuable feed resource for both monogastric and ruminant animals if appropriately harnessed.

The mineral and mineral ion composition of rubber seed cake is as shown in table 2 below. The calcium content obtained for RSC (1.82g/kg) in this study is within the range of values (1.5 – 3.9 g/kg) for deoiled rubber seed meal reported by Heuze and Tran, (2011); Jayasuriya *et al.* (1982); Narahari *et al.* (1984). Nwokolo and Akpakunam, (1986) had reported RSC to contain 2.05 g/kg calcium. The calcium composition of RSC (0.18%) is comparable to that of wheat offal (0.10 – 0.16%), palm kernel cake (0.21 – 0.25%), BDG (0.20 – 0.32%), and cassava meal (0.15 - 0.20%) and superior or more than those of maize (0.01 – 0.03%), guinea corn (0.04 – 0.05%), millet (0.02 – 0.06%), rice bran (0.04 – 0.06%), and lower than the calcium content of fish and meat meal (McDonald *et al.*, 1995; Aduku, 1993).

Table 1: The proximate (g/100gDM) and gross energy (Kcal/kg) composition of Rubber seed cake

Parameters	Mean ± SD	SEM	CV (%)
Dry matter	90.77 ± 0.058	0.03	0.60
Crude Protein	14.37 ± 0.15	0.09	1.06
Crude fibre	3.70 ± 0.10	0.06	2.70
Ash	4.43 ± 0.115	0.07	2.605
Ether Extract	16.87 ± 0.15	0.09	0.91
NFE	60.63 ± 0.2	0.10	0.30
CHO (NFE + CF)	64.33 ± 0.3	0.10	0.40
Gross Energy (Kcal/kg)	2,141.01 ± 6.0	3.00	0.00

SD = Standard deviation; SEM = Standard error of mean; CV = Coefficient of variation.

The phosphorus content (measured as phosphate ions) of RSC in this study (1.25g/kg) is lower than the range of values (3.4 – 7.5g/kg) reported by Heuze and Tran, (2011) but higher than 0.03 - 0.08% reported for cassava meal (McDonald *et al.*, 1995; Aduku, 1993) and comparable with those of millet (0.10 -0.03%), palm kernel cake (0.16 - 0.74%), maize (0.09 – 0.29%). Calcium and phosphorus are essential for bone, legs, skull, teeth, formation, growth fertility and development in farm animals.

The iron content of (0.0827g/kg) compares favourably with the findings of Nwokolo and Akpakunam, (1986) who reported rubber seed meal to contain 0.05g/kg iron. Iron (Fe) is essential in the body of animal as haem component of the blood which assists in distribution of oxygen and removal of CO_2 from the system.

The manganese and zinc composition of RSC (4.7×10^{-4} and 5.6×10^{-3} g/kg respectively) obtained in this study were lower than the values (0.035 and 0.5 g/kg) earlier reported by Nwokolo and Akpakunam, (1986) for RSC. Manganese and zinc are constituents of important enzymes in the body and their deficiency leads to poor growth, leg deformities, poor fertility, frequent abortion, reduced hatchability, perosis or slipped tendon in chicks, or depressed appetite, poor feed efficiency, parakeratosis, subnormal growth among others.

The magnesium and potassium content of RSC studied (0.342 and 0.34g/kg respectively) were lower than the corresponding values (12.0 and 7.20 g/kg respectively) reported by Heuze and Tran (2011). A higher value for magnesium (5.0g/kg) was reported by Nwokolo and Akpakunam, (1986) for the same ingredient. Magnesium acts as enzyme activator especially in transferases decarboxylases and acyl transferases. Its deficiency can lead to hypomagnesemic tetany in animals. Other minerals found in RSC at lower levels include sodium (286.7 ± 2.90g/100gDM) selenium (1.0×10^{-4} g/kg), lead (1.0×10^{-4} g/kg), sulphate ions (1.25g/kg). Level of lead obtained in this study was very low and would not be deleterious if fed to livestock.

Carotenoids necessary for vision, epithelial cells and bone formation could not be detected in RSC.

The screening for anti-nutritional factors and their composition in RSC is as shown in table 3 below. The screening revealed that RSC contained saponin, tannin, oxalate, phenol, sterioids and flavonoid. Terpenoids and cyanogenetic glycosides were not detected. The results of the quantitative analysis of RSC indicated that it contained (mg/100g) phytates (45.0); saponins (160.0); tannins (12.0); oxalate (25.0); phenol (16.83); steroids (86.67); and flavonoid (125.0). Saponin had the highest value (160 mg/100g) followed by flavonoid and steroid which is about 0.16, 0.125 and 0.087% respectively. The values obtained for these anti-nutritional factors will have no deleterious effect on animals if RSC is used in formulating their diet since it will not constitute up to 70% of the feed composition.

Table 2: Minerals and carotenoid composition of rubber seed cake (mg/100g)

Parameters	Mean ± SD	SEM	CV (%)
Calcium (Ca)	181.7 ± 3.0	2.0	2.0
Iron (Fe)	8.27 ± 0.15	0.09	1.85
Potassium (K)	34.10 ± 0.9	0.50	2.50
Magnesium (Mg)	34.17 ± 1.44	0.83	4.22
Manganese (Mn)	0.047 ± 0.01	0.003	12.37
Sodium (Na)	286.7 ± 2.90	1.70	1.00
Lead (Pb)	0.01 ± 0.001	0.001	0.01
Zinc (Zn)	0.567 ± 0.058	0.03	10.19
Selenium (Se)	0.01 ± 0.001	0.001	0.001
Phosphate ions	125 ± 1.00	0.58	0.80
Sulphate ions	23.0 ± 1.00	0.58	4.35
Carotenoids (mg/100g)	ND	ND	ND

ND = Not detected; SD = Standard deviation; SEM = Standard error of mean; CV = Coefficient of variation.

Table 3: Phytochemical or anti-nutritive factor composition of rubber seed cake (mg/100g)

Parameters	Qualitative analysis	Mean ± SD	SEM	CV (%)
Phytates	NT	45.0 ± 5.00	2.89	11.11
Saponins	+	160.0 ± 8.66	5.00	5.41
Tannins	+	12.0 ± 0.866	0.50	7.22
Oxalates	+	25.0 ± 1.0	0.58	4.00
Phenols	+	16.83 ± 1.04	0.60	6.18
Steroids	+	86.67 ± 7.64	4.41	8.81
Flavonoids	+	125 ± 1.00	1.00	1.00
Terpenoids	-	ND	-	-
Cyanogenetic Glycosides	-	ND	-	-

SD = Standard deviation; SEM = Standard error of mean; CV = Coefficient of variation; NT = Not tested; ND = Not detected.

Phytates if present in large quantity can bind minerals like phosphorus, zinc and iron and reduce their absorption. Saponins are bitter, insoluble in ether but soluble in water and give a glycones that are poisonous and can cause haemolysis of blood and are known to cause cattle poisonoing (Kar, 2007). Saponins are also important therapeutically as they are shown to have hypolipidemic and anti-cancer activity by neutralizing some cancer causing enzymes in the gut (Doughari, 2012). Higher contents of tannin forms complexes with proteins, carbohydrates, gelatin and alkaloids and decreases their digestibility and absorption in animals. Tannin rich plants have medicinal usefulness of being used for the treatment of diseases like leucorrhoea, rhinnorhoea and diarrhea (Doughari, 2012). Tannin may also provide protection against microbial degradation of dietary proteins in the rumen (Aletor, 1993; Erukainure *at al*., 2011). High levels of oxalate had been shown to cause stomach ache and sandy feeling in the mouth, acridity or irritation. Phenols and flavonoids have been indicated to represent a host of natural anti-oxidants and help to decrease cancer risk, urinary tract problems and improve memory function and healthy aging as they protects cells from inflammation and oxidation (Doughari, 2012, Purdue University, 2015). Plant steroids or steroid glycosides or cardiac glycosides have therapeutic applications as arrow poisons and cardiac drugs (Firn, 2010). It promotes nitrogen retention in osteoporosis and in animals with wasting illness (Madziya *et al*., 2010; Mauriya *et al*., 2008).

CONCLUSION

- Based on the proximate, energy, mineral and anti-nutritional factor composition of the presently less utilized Nigeria rubber seed cake but is widely available in southern Nigeria has a promise as feed ingredient for use in livestock production by local and large scale farmers without imparting any harmful effect on the animals and their products. .

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