

# THE EFFECTS OF PROCESSED RUBBER (*Hevea brasiliensis*) SEED MEAL ON THE CHEMICAL COMPOSITION OF RATION, FEED INTAKE AND NUTRIENTS DIGESTIBILITY IN RABBITS (*Oryctolagus cuniculus*)

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↳ Supporting Information

**ABSTRACT:** This study aimed at evaluating the influence of processed rubber (*Hevea brasiliensis*) seed meal (RSM) on the chemical composition of feed, feed intake and nutrients digestibility in rabbits. For the purpose, 30 female (local breed) rabbits with an average weight of  $2.7 \pm 0.12$  kg were randomly allotted to five dietary treatments (6 rabbits in 1 treatment). The feeding trial lasted for four weeks and the various rations were: negative control ration, not containing rubber seeds (R0-) and 4 different rations each containing 20% of the rubber seed meal (raw, toasted, soaked, and boiled respectively for R0+, R1, R2, and R3 rations). The rubber seed meal, faeces and feed sample were sun dried to constant weight, urine was also collected and a sample was used for chemical analysis. The results showed that the boiled seeds RSM exhibited the highest crude protein content (20.61% DM) and the low concentration of anti-nutritional factors (ANFs) (21.83 mg/kg HCn; 30.53%; 21.26% and 3.67% respectively for hydrogen cyanide (HCn), phenols, flavonoids and tannins) compared to seeds from other treatments. Animals receiving the ration with boiled rubber seed meal (BRSM) at 100 °C for 45 minutes (R3) showed better feed intake and best rate of digestive utilization coefficients of dry matter, organic matter, crude protein and crude fibres 32.24%; 38.55%; 61.03% and 80.58% respectively. The ration containing the boiled rubber seeds for 45 min resulted in better nutrient intake and the best digestive utilization coefficients in rabbits.

**Keywords:** Digestibility, *Hevea brasiliensis*, Nutritional value, Rabbit, Rubber seed.

**Abbreviations:** UDTRF: University of Dschang Teaching and Research Farm; ANPROLA: Animal Production and Nutrition Laboratory; SOCAPALM: Cameroon Company of Palm Groves; RRSM: raw rubber seed meal. DM: Dry Matter, OM: Organic Matter, CP: Crude Protein, CF: Crude Fibre. T0: Ration not containing rubber seeds meal, RRSM: Control ration with 20% untreated rubber seed meal, BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes, SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours, TRSM: ration with 20% toasted rubber seed meal for 45 minutes; ANOVA: Analysis of variance; SPSS: Statistical Package for Social Sciences.

## INTRODUCTION

Food remains the major challenge for successful animal breeding (Niba et al., 2012). It represents 60-70% of production costs in intensive farming (Defang et al., 2014). This could be explained by the regular increase in the price of conventional feedstuffs such as soybeans or soybean meal which is imported, and competition between human and livestock for this primary product. In this situation, some breeders in developing countries tend to reduce the incorporation rate of expensive and scarce ingredients in ration formulation, which could lead to an unbalanced feed and consequently to a poor expression of the genetic potential of animals. According to Akinmutimi (2007), the consequences of the above are the reduction in the animal protein production and then, insufficient animal protein consumption, due to the scarcity and high cost of animal products. The attention in animal nutrition in recent years has been centred mostly on the search for alternative feed ingredients which are cheaper, readily available, have potential to replace expensive conventional feedstuffs during period of shortage and has little or no competition with human dietary demand (Alao et al., 2017; Sugiharto et al., 2018).

Rubber seeds (*Hevea brasiliensis*) appear as one of the alternative non-conventional feedstuff of interest (Iyayi et al., 2008). In Cameroon, rubber is produced for exportation and seeds are usually discarded causing environmental hazard. Rubber seeds are produced by the rubber tree (*Hevea brasiliensis*), belonging to the family of Euphorbiaceae. It is known and used for its latex, but its seeds can be used in animal feeding. Rubber seed is a capital non-conventional feedstuff, which has received scant research attention in fish feeding (Sharma et al., 2014; Suprayudi et al., 2015; Deng et al., 2015), layers (Atchibri et al., 2008), pregnant and lactating pigs (Koné et al., 2016), chickens (Aguihe et al., 2017) and ducks (Amanidja et al., 2019). Researchers have revealed that dried seeds of *Hevea brasiliensis* contain between 17- 25% protein (Sharma et al., 2014), 50.2% fat, 6.5% crude fibre, 3.6% ash and 18.2% carbohydrate, as well as reasonable

amounts of mineral trace elements (Udo et al., 2018). However, variability in nutrient content will depend on the variety, age at harvesting, soil type, processing methods and storage of the by-product. Despite the acceptable protein rate, rubber seed meal is characterized by the presence of anti-nutritional factors (ANFs), specially cyanogen glycosides, whose metabolic end product is hydrogen cyanide, that impacts negatively the physio-metabolic responses (Sharma et al., 2014; Deng et al., 2015). Freshly harvested rubber seeds contain anti-nutritional factors such as tannins (0.07%), oxalate (0.18%), saponins (0.76%) and phytate (0.51%) and a toxic element that can create disorders when they are immediately introduced into animal feed; also it contributes to gastrointestinal issues and reduce metabolic activity when ingested (Thangaraj, 2016). It is the hydrocyanic acid (Syahrudin et al., 2014; Ahaotu, 2018), whose rate is 24.89 % (Udo et al., 2018), similar to cassava cyanic acid (Udo et al., 2018). Hence, the processing of rubber seeds could reduce their anti-nutritional factors and consequently optimize their use in animal feed. It is with this in mind that this work was initiated in order to contribute to the valorisation of agricultural by-products in animal feeding. Specifically it was to evaluate the influence of processing rubber seeds on the anti-nutritional factor contents, nutritional values, chemical composition of the ration; feed intake and nutrients' digestibility in rabbits fed with rations containing seeds subjected to different treatments.

## MATERIALS AND METHODS

### Study site

The study was conducted at the rabbitry unit of the University of Dschang Teaching and Research Farm (UDTRF) and in the Animal Production and Nutrition Laboratory (ANPROLA) of the Dschang University, and under Ethical regulation of Dschang University for animal welfare in *in vivo* studies. The UDTRF is located between 5° 44'-05° 36' and 5° 44'-05° 37' North latitude and 10° 06'-09° 94' and 10° 06'-09° 85' East longitude, at an altitude of 1420 m from the West Cameroon Region. The mean annual rainfall is about 2000 mm, the wet season last from march to November and the dry season from late November to early March.

### Experimental rabbits and housing and prophylaxis

A total of 30 female (local breed) rabbits aged 5 - 6 months were used for the trial. The rabbits were managed intensively and housed individually in specially constructed metabolic cages (Californian type) measuring 60 cm × 40 cm × 25 cm in dimension. The adaptation period lasted ten days, during which the rabbits were treated against parasitic infection with ivermectin® (0.2 ml / kg body weight) subcutaneous and multivitamin added to their water.

### Experimental design and management

For the digestibility trial, 30 rabbits were randomly allotted to each of the 5 dietary treatments (6 rabbits per treatment). Each of the rabbit formed an experimental unit. The experiment lasted for seven days. The rabbits had free access to feed and potable water.

### Plant material

The rubber seeds were harvested in the rubber plantations of SOCAPALM® (Cameroon Company of Palm Groves) in the Moungo Division, Littoral Region. They were decorticated, separated into 4 batches and processed differently. First batch was sun dried, second batch was cooked, third batch was toasted and the fourth batch was soaked. The processed rubber seeds were separately hammer-milled prior to experimental diet formulation to produce the respective meals as raw rubber seeds meal (RRSM), soaked (SRSM), boiled RSM (BSRM) and toasted RSM (TRSM).

### Processing methods

#### Boiling/cooking

Raw decorticated rubber seeds were put into a cooking pot containing boiling water. The content was allowed to boil for 45 minutes before the seeds were descanted. The boiled seeds were sun dried, milled and stored for further use.

#### Toasting

Raw decorticated rubber seeds were toasted in a metallic frying pan for 45 min. After cooling, they were sun dried, milled and stored for further use.

#### Soaking

Raw decorticated rubber seeds were soaked in water at the ambient temperature, inside a closed inoxidable metal drum for 72 hours, followed by draining. The soaked seeds were sun dried, milled and stored for further use.

### Chemical analyses

Chemical compositions of the processed rubber seed meals were analysed using the methods of AOAC (2000). The determinations of some minerals' contents were carried out according to the methods described by Pauwels et al. (1992). While the anti-nutritional factors were evaluated: the cyanide contents as described by ISO 6703-2 (1984); tannins content by the Folin-Ciocalteu method (Govindappa et al., 2011); the content of total phenols by the method described by (Ramdé-Tiendrébéogo et al., 2012); the total flavonoid content using the aluminium chloride colorimetric method (Chang et al., 2002).

### Experimental diets

Five experimental diets were formulated to meet the nutritional requirements of the rabbits. T0 (negative control) diet was free of rubber seed meal, RRSM (positive control) contained 20 % untreated RSM, while, BRSM, SRSM and TRSM respectively containing 20% of treated rubber seeds meal as presented in Table 1.

**Table 1 - Percentage composition and proximate analysed experimental rations.**

Experimental rations (%)	TO (0)	RRSM (20)	BRSM (20)	SRSM (20)	TRSM (20)
<b>Ingredients (% MS)</b>					
Maize	37	25	25	25	25
RRSM	0	20	0	0	0
Treated RSM	0	0	20	20	20
Wheat brand	20	20	20	20	20
Soybean meal	4	1	1	1	1
Fish meal	3	1	1	1	1
Palm kernel cake	11	11	11	11	11
Cotton seed cake	4	1	1	1	1
<i>Trypsacum laxum</i>	18	18	18	18	18
Sea shell	1	1	1	1	1
Palm oil	1	1	1	1	1
Salt	0.5	0.5	0.5	0.5	0.5
Premix 0.5 *	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100
<b>Analysed chemical compositions of rations</b>					
Dry matter (%)	94.22	95.06	96.33	95.57	96.50
OM (% DM)	85.79	87.35	90.69	90.60	90.58
Ash (% DM)	8.64	7.71	5.71	4.85	5.99
CP (% DM)	16.89	16.70	17.42	16.90	16.65
ME (Kcal/Kg)	2659.12	2745.53	2773.96	2759.38	2753.45
CF (% MS)	12.14	12.67	13.71	13.38	12.95
* Composition of the 5% premix: Vit A: 3.000.000 IU, Vit D: 50.0000 IU, Vit E: 6.000 mg, Vit K: 600 mg, Vit B1: 600 mg, Vit B2: 800 mg, Vit B3: 1800 mg, Vit B6: 400 mg, Vit12: 6 mg, folic acid: 250 mg, Niacin: 600 mg, Cl: 86.500 mg, Fe: 12.000 mg, Cu: 1200 mg, manganese: 12.000 mg, Zn: 10.000 mg, I: 100 mg, Se: 40 mg, magnesium: 3397 mg, Na: 283 mg, CA: 215.166 mg, Methionine: 130.000 mg, lysine: 50.000 mg. RRSM: raw rubber seed meal. DM= Dry Matter, OM= Organic Matter, CP= Crude Protein, CF= Crude fiber. TO: Ration not containing rubber seeds meal, RRSM: Control ration with 20% untreated rubber seed meal, BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes, SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours, TRSM: ration with 20% toasted rubber seed meal for 45 minutes.					

### Data collection

#### Metabolic cage trial

During the experimental period, excreta and urine were collected daily for seven days. Faeces samples were pooled, weighed, dried, weighed again and ground. The nutrient composition of the faeces and urine samples was determined according to the procedures described by AOAC (2000). The resulting data were used to calculate the apparent nutrient digestibility coefficients.

#### Statistical analysis

Collected data were subjected to a one ways analysis of variance (ANOVA), using SPSS 20.0 software. Where there was a significant difference, Duncan's test at 5% significance was used to separate the means.

## RESULTS

The effects of processing methods on anti-nutritional factors in raw and processed rubber seed meal (RSM) are presented on Table 2. Generally, processing had significant ( $p < 0.05$ ) effects on ANFs. The ANFs in the boiled RSM was significantly ( $p < 0.05$ ) lower compared to other treatments. However, flavonoids content (21.26%) for BRSM remained comparable ( $p > 0.05$ ) to that of TRSM (22.46%).

#### Effects of processing methods on proximate composition of raw and processed rubber seeds meals

Table 3 presents the effects of processing methods on proximate composition of raw and processed rubber seeds meals. It appears that with the exception of toasting RSM, the treatments significantly ( $p < 0.05$ ) improved the composition of the seeds meal. Boiled RSM had significantly ( $p < 0.05$ ) higher results but its fat content and energy was the lowest. Toasted RSM despite the fact that its content in other nutrient components was significantly ( $p < 0.05$ ) lower compared to untreated seeds, had the highest fat and energy contents.

#### Effects of processing methods on minerals composition of raw and processed rubber seeds meal

Table 4 shows the effects of processing methods on the minerals composition of raw and processed rubber seeds meal. Generally, the mineral contents in the processed RSM was significantly ( $p < 0.05$ ) influenced by processing methods compared to the raw RSM. For macro minerals (Ca, K and P) boiled seeds meal had significantly ( $p < 0.05$ ) higher values than that of the others treatment.

#### Effects of processing methods of rubber seeds meal on feed intake in rabbits

Table 5 presents the effects of rubber seeds treatments on rabbit feed intake. The ingestion of nutrients had significantly ( $p < 0.05$ ) increased with the addition of rubber seeds in the ration compared to the TO ration (not containing rubber seeds). Nevertheless, feed ingestion of the dry matter and organic matter in animals fed RRSM diet was significantly lower ( $p < 0.05$ ) than that of batch TO. The BRSM showed the significantly ( $p < 0.05$ ) higher values.

**Table 2 - Effects of processing methods on anti-nutritional factors in rubber seeds meal.**

Anti-nutritional factors (ANFs)	Processed rubber seeds' meals				SEM	P
	RRSM	TRSM	SRSM	BRSM		
H <sub>2</sub> CN(mg/kg H <sub>2</sub> CN)	87.34±0.00 <sup>a</sup>	44.39±1.02 <sup>b</sup>	30.57±2.06 <sup>c</sup>	21.83±0.00 <sup>d</sup>	2.12	0.001
Phenol (%)	90.71±0.07 <sup>a</sup>	68.29±0.61 <sup>b</sup>	43.53±0.10 <sup>c</sup>	30.53±0.03 <sup>d</sup>	6.97	0.01
Flavonoids (%)	45.47±0.15 <sup>a</sup>	22.46±0.12 <sup>c</sup>	43.14±1.10 <sup>b</sup>	21.26±0.03 <sup>c</sup>	3.39	0.001
Tanins (%)	16.04±0.04 <sup>a</sup>	10.55±0.04 <sup>b</sup>	5.63±0.07 <sup>c</sup>	3.67±0.02 <sup>d</sup>	1.44	0.001

<sup>a, b, c</sup> Mean values on the same row with different superscripts are significantly different (P<0.05) SEM: standard error on mean; p: probability. RRSM: Raw Rubber Seed Meal; TRSM: Toasted Rubber Seed Meal; SRSM: Soaked Rubber Seed Meal; BRSM: Boiled Rubber Seed Meal; H<sub>2</sub>CN: hydrogen cyanide.

**Table 3 - Effects processing methods on analysed proximate composition of raw and treated rubber seed meal (RSM).**

Parameters	Processed rubber seeds' meals				SEM	P
	RRSM	TRSM	SRSM	BRSM		
DM (%)	92.20±0.20 <sup>b</sup>	89.20±0.20 <sup>c</sup>	96.43±0.20 <sup>a</sup>	92.00±0.01 <sup>b</sup>	0.79	0.001
OM (%)	96.50±0.01 <sup>b</sup>	95.68±0.09 <sup>c</sup>	96.17±0.02 <sup>b</sup>	97.53±0.07 <sup>a</sup>	0.23	0.001
CP (%)	19.53±0.20 <sup>b</sup>	18.39±0.20 <sup>c</sup>	19.80±0.09 <sup>b</sup>	20.61±0.15 <sup>a</sup>	0.26	0.001
CF (%)	11.24±0.02 <sup>d</sup>	14.99±0.04 <sup>c</sup>	17.32±0.02 <sup>b</sup>	18.90±0.05 <sup>a</sup>	0.87	0.001
Fat (%)	37.04±0.08 <sup>c</sup>	48.94±0.10 <sup>a</sup>	42.17±0.03 <sup>b</sup>	27.50±0.05 <sup>d</sup>	2.36	0.001
Ash (%)	3.50±0.01 <sup>c</sup>	2.31±0.09 <sup>d</sup>	3.80±0.05 <sup>b</sup>	4.43±0.07 <sup>a</sup>	0.23	0.001
ME (Kcal)	4526.61±1.90 <sup>c</sup>	4835.57±1.84 <sup>a</sup>	4826.29±0.05 <sup>b</sup>	3939.44±0.61 <sup>d</sup>	1.78	0.001

<sup>a, b, c, d, e</sup> the means bearing the same letters in the same row are not significantly different at the threshold of 5; SEM: standard error on mean; p: probability; DM: dry matter; OM: organic matter; CP: crude protein; CF: crude fibre; RRSM: Raw Rubber Seed Meal; TRSM: Toasted Rubber Seed Meal; SRSM: Soaked Rubber Seed Meal; BRSM: Boiled Rubber Seed Meal.

**Table 4 - Effects of processing methods on the mineral composition of raw and processed rubber seeds meal.**

Minerals (%)	Processed rubber seeds' meals				SEM	P
	RRSM	TRSM	SRSM	BRSM		
Ca <sup>2+</sup>	0.09±0.01 <sup>b</sup>	0.04±0.01 <sup>c</sup>	0.02±0.00 <sup>d</sup>	0.11±0.01 <sup>a</sup>	0.011	0.001
Mg <sup>2+</sup>	0.29±0.03 <sup>a</sup>	0.04±0.01 <sup>c</sup>	0.27±0.02 <sup>a</sup>	0.18±0.04 <sup>b</sup>	0.030	0.001
P	0.24±0.01 <sup>b</sup>	0.08±0.00 <sup>d</sup>	0.20±0.01 <sup>c</sup>	0.32±0.01 <sup>a</sup>	0.025	0.001
K <sup>+</sup>	0.23±0.00 <sup>b</sup>	0.15±0.01 <sup>c</sup>	0.24±0.01 <sup>b</sup>	0.29±0.01 <sup>a</sup>	0.015	0.001
Na <sup>2+</sup>	0.02±0.00 <sup>a</sup>	0.01±0.00 <sup>b</sup>	0.02±0.00 <sup>a</sup>	0.02±0.00 <sup>a</sup>	0.001	0.001

<sup>a, b, c, d, e</sup> the means bearing the same letters in the same row are not significantly different at the threshold of 5%. RRSM: Raw Rubber Seed Meal; TRSM: Toasted Rubber Seed Meal; SRSM: Soaked Rubber Seed Meal; BRSM: Boiled Rubber Seed Meal; SEM: standard error on mean; p: probability; Ca<sup>2+</sup>: calcium, Mg<sup>2+</sup>: magnesium, P: phosphorus, K<sup>+</sup>: potassium, Na<sup>2+</sup>: sodium.

**Table 5 - Effects of processed rubber seed meal on rabbit feed intake.**

Feed intake	T0	Processed rubber seeds' meals				SEM	P
		RRSM	TRSM	SRSM	BRSM		
DM (g)	114.90±0.29 <sup>d</sup>	110.35±0.67 <sup>e</sup>	116.67±0.12 <sup>c</sup>	118.53±0.36 <sup>b</sup>	125.17±0.39 <sup>a</sup>	1.30	0.001
OM (g)	108.46±0.26 <sup>c</sup>	102.85±0.69 <sup>d</sup>	109.77±0.31 <sup>b</sup>	110.27±0.34 <sup>b</sup>	121.64±0.41 <sup>a</sup>	1.63	0.001
CP (g)	19.24±0.31 <sup>d</sup>	20.33±0.29 <sup>c</sup>	20.58±0.70 <sup>c</sup>	21.10±0.59 <sup>b</sup>	22.01±0.13 <sup>a</sup>	0.24	0.001
CF (g)	16.33±0.20 <sup>c</sup>	18.10±0.19 <sup>b</sup>	18.90±0.37 <sup>a</sup>	18.98±0.19 <sup>a</sup>	19.06±0.30 <sup>a</sup>	0.28	0.001

<sup>a, b, c, d, e</sup> the means bearing the same letters in the same row are not significantly different at the threshold of 5%; SEM: standard error on mean; p: probability; DM: dry matter; OM: organic matter; PB: crude protein; CB: cellulose brute. T0: Ration not containing rubber seeds meal; RRSM: Control ration with 20% untreated rubber seed meal; TRSM: ration with 20% toasted rubber seed meal for 45 minutes; SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours; BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes.

### Effects of processing methods of rubber seeds' meal on the apparent digestibility of nutrients in rabbits

Table 6 summarises the effects of processed rubber seeds meal on the nutrients digestibility in rabbits. In general, there were significant (p<0.05) differences in the apparent digestibility coefficient (ADC) of DM, OM, CP and CF on the control diets. Additionally, the apparent digestibility coefficient of DM, MO, CP significantly (p<0.05) increased for the rations containing processed RSM (TRSM, SRSM and BRSM) compared to the control ration (T0) and that containing untreated rubber seeds (RRSM). However, the apparent digestibility coefficient of all nutrients decreased significantly (p<0.05) for the RRSM ration except that of crude protein which remained comparable to that of the T0 ration.



### Effects of processing methods of rubber seeds on nitrogen balance

Table 7 shows the effects of processing rubber seeds meal on the apparent digestibility of nutrients and nitrogen balance. Processing significantly ( $p < 0.05$ ) influenced ingestion and digestibility of nitrogen. Ration containing the boiled RSM had the better results however the nitrogen content ingested was comparable among rations. The apparent digestibility of nitrogen was better with the ration containing boiled rubber seeds (30.29%).

**Table 6 - Effects of processed rubber seed meal on the apparent digestibility of nutrients in rabbits.**

ADC (%)	T0	Processed rubber seeds' meals				SEM	P
		RRSM	TRSM	SRSM	BRSM		
DM	21.16±0.14 <sup>c</sup>	18.90±0.67 <sup>d</sup>	26.79±0.26 <sup>b</sup>	28.80±0.36 <sup>b</sup>	32.24±2.47 <sup>a</sup>	1.33	0.001
OM	18.15±1.33 <sup>c</sup>	14.68±0.26 <sup>d</sup>	31.10±0.73 <sup>b</sup>	32.34±0.45 <sup>b</sup>	38.55±1.93 <sup>a</sup>	2.42	0.001
CP	55.02±2.48 <sup>b</sup>	56.07±0.21 <sup>b</sup>	55.46±0.80 <sup>b</sup>	60.30±1.07 <sup>a</sup>	61.03±1.81 <sup>a</sup>	0.75	0.001
CF	64.86±1.83 <sup>b</sup>	53.84±1.51 <sup>d</sup>	57.63±2.71 <sup>c</sup>	80.57±0.11 <sup>a</sup>	80.58±0.67 <sup>a</sup>	3.02	0.001

a, b, c, d, e: the means bearing the same letters in the same row are not significantly different at the threshold of 5%; SEM: standard error on mean; p: probability; ADC: apparent digestibility coefficient; DM: dry matter; OM: organic matter; CP: crude protein; CF: Crude fiber. T0: Ration not containing rubber seeds meal; RRSM: Control ration with 20% untreated rubber seed meal; TRSM: ration with 20% toasted rubber seed meal for 45 minutes; SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours; BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes.

**Table 7- Effect of processed rubber seeds meal on the digestive system of nitrogen.**

Nitrogen (N) balance (g/J)	T0	Processed rubber seeds' meals				SEM	P
		RRSM	TRSM	SRSM	BRSM		
Intake nitrogen	2.51±0.13 <sup>a</sup>	2.45±0.17 <sup>a</sup>	2.52±0.05 <sup>a</sup>	2.60±0.53 <sup>a</sup>	2.62±0.03 <sup>a</sup>	0.02	0.34
Faecal nitrogen	1.28±0.02 <sup>c</sup>	1.05±0.19 <sup>d</sup>	1.52±0.08 <sup>b</sup>	1.59±0.07 <sup>ab</sup>	1.77±0.11 <sup>a</sup>	0.07	0.001
Urinary nitrogen	0.41±0.02 <sup>c</sup>	0.43±0.01 <sup>c</sup>	0.53±0.02 <sup>b</sup>	0.38±0.05 <sup>c</sup>	0.64±0.06 <sup>a</sup>	0.02	0.001
Retention nitrogen	0.73±0.05 <sup>a</sup>	0.36±0.01 <sup>d</sup>	0.46±0.02 <sup>c</sup>	0.62±0.01 <sup>b</sup>	0.74±0.04 <sup>a</sup>	0.04	0.001
ADC (%)	29.25±1.65 <sup>a</sup>	13.16±0.31 <sup>d</sup>	18.20±0.54 <sup>c</sup>	24.07±0.31 <sup>b</sup>	30.29±0.72 <sup>a</sup>	1.69	0.001

a, b, c, d, e: the means bearing the same letters in the same row are not significantly different at the threshold of 5%; SEM: standard error on mean; p: probability; T0: Ration not containing rubber seeds meal; RRSM: Control ration with 20% untreated rubber seed meal; TRSM: ration with 20% toasted rubber seed meal for 45 minutes; SRSM: ration with 20% rubber seed meal soaked in water at room temperature for 72 hours; BRSM: Ration with 20% rubber seed meal soaked in boiling water for 45 minutes.

## DISCUSSION

The HCn content of the fresh RSM (87.34 mg/kg) was lower than 186; 415.10 and 315.89 mg/kg values reported by Eka et al. (2010), Sharma et al. (2014) and Aguihe et al. (2017) respectively. This result supports the report of (JECFA, 1993) that genetic and environmental factors, location, season, and soil factors are reasons for the wide variations observed in the ANFs. Moreover, reduction in the level of ANFs was effective with the adopted processing methods and this revealed that cyanide, tannins, phenol and flavonoids level in the raw seeds decreased for soaked, toasted, and boiled processing techniques. The highest level of reduction was obtained in the boiled seeds followed by soaked seeds and this is in agreement with previous researchers (Sharma et al., 2014) who stated that heat treatments and fermentation tends to reduce the concentration of ANFs in RRSM and makes them nutritionally less active. The finding corresponds with the description that anti-nutritional factors are heat liable (Eka et al., 2010; Aguihe et al., 2017; Udo et al., 2018) factors, this could be explained by the fact that the heat would have destroyed part of the ANFs and the water cooking would have washed out other ANFs. This result corroborates with that of Ogundipe et al. (2008), which showed that 71.91% of tannin is destroyed after 30 minutes of boiling the seeds by *Mucuna*. Similarly Chakam (2006) concluded that cooking is the best treatment for detoxify cowpea seeds. Soaked seeds take second place for this low content, then follow the toasted seeds. This could be explained by the unique action of heat on the one hand (toasting) and water on the other hand (soaking) or in the treatment by boiling the two factors interact.

The crude protein (CP) value of the raw rubber seed (19.53% DM) obtained in this study was in agreement with the results of Lalabe (2017) but higher than 17.41 % as reported by Eka et al. (2010). The crude fibre content (11.24%) obtained in this trial is in agreement with that of Nwokolo (1988) (10.3% DM), but significantly higher than that of Aguihe et al. (2017) and Udo et al. (2018) who obtained 4.40; 5.61; 5.88% DM respectively. The variations observed could be attributed to the differences in variety of rubber trees, soil composition, climatic conditions as well as agricultural practices or plan mechanism meant for absorption of nitrogenous nutrients and inadequate fertilizer application of the rubber plantation (Chanjula et al., 2010). Also it can be explained by the processing and analysis methods used by these authors. The boiling of rubber seeds exhibited the greatest crude protein content while that the toasting presented the smallest value, this corroborates the results of Udo et al. (2018) who reported an increase in crude protein content when

rubber seeds were boiled and a reduction in this content when toasted. The differences could be due to the effect of heat, which denatured part of the proteins during the two treatments.

The minerals contents of the raw seed were lower than that of Udo et al. (2018) with 1740 and 2066 mg/kg for calcium and magnesium respectively. Likewise, the minerals content of raw rubber seeds meal (RRSM) is lower than that obtained by Far et al. (2019). These differences may be attributed to genetic variations, as well as climate, environmental and geographical factors. The increase in minerals after boiling would be due to the fact that the minerals trapped by the anti-nutritional factors were released by heat. This result is in agreement with that of Udo et al. (2018) and that of Far et al. (2019) who both noted an increase in the minerals when boiling and a reduction in these when toasting the rubber seeds. The best results were registered with the boiled RSM with regard to the calcium, potassium, sodium and phosphorus contents and with the treatments SRSM and RRSM for magnesium.

Intake of nutrients were significantly higher for rabbits fed boiled RSM this could be explained by the fact that the boiling time was an effective treatment to reduce trypsin inhibitors, cyanide, saponins, and tannins (Chakam, 2006). The result of this trial is in agreement with the findings of Aguihe et al. (2017) and Far et al. (2019) who noted a better intake of the ration containing boiled rubber seeds meal.

The apparent digestibility coefficient of dry matter (DM) and organic matter (OM) were significantly higher for rabbits fed diet containing boiled rubber seed meal compared to the rest of the treatments. These results are lower than those of Lounaouci-Ouyed (2014), who obtained a load digestive factor from dry matter (79.8%; 75.8%; 78.2%) and organic matter (79.2%; 75.6% and 78.0%), when rabbits were fed soybeans, peas and faba bean as sources of protein respectively. Likewise Cunha (1993) obtained a digestive utilization coefficient of the dry matter of 60.9% and an apparent digestibility coefficient of the organic matter of 61.4% when soybean was replaced at 20% by *Vicia benchalensis* in the rabbit ration. Indeed, these results could be due to the effects of the chemical composition of the ration, sex, age and breed, since digestibility depends on these factors. In fact, Cunha (1993) worked with animals of 3 months, of New Zealand breed. While in this study the animals used were 5 to 6 months. The apparent digestibility coefficient of crude protein (CP) and crude fibre (CF) were comparable for rabbits fed with RRSM soaked in water at room temperature for 72 hours but significantly higher compared with the rest of the treatment. The results obtained within these framework were lower than those obtained by Aguihe et al. (2017) (72.54%) when broilers were fed a ration containing boiled rubber seeds; but comparable to those of Wafar et al. (2017), who obtained a digestibility coefficient of (60.45%) when rabbits were fed raw kapot tree seeds. The best coefficient of digestibility of crude cellulose was obtained with the ration containing the boiled rubber seeds meal (BRSM), a result comparable to that of Wafar et al. (2017) (58.14%), but higher than that of Aguihe et al. (2017) (43.47%). This difference could be due to the chemical composition of the ration and the animal species, which agrees with Rivière's assertion (Rivière, 1991), which showed that feed and animal species strongly influence digestibility of nutrients.

This apparent digestibility coefficient of nitrogen was comparable to that of ration R0- not containing rubber seeds (29.25%); but higher than that of the ration containing untreated seeds (13.16%). This could be explained by the presence of anti-nutritional factors in the latter that would have interfered with digestive use nitrogen.

## CONCLUSION

The results of the present research showed that boiling rubber seed is effective in reducing anti-nutrients factors in rubber seed. Also, the contents of dry matter, organic matter, proteins, crude fibre, ash and energy increased with treatment boiling rubber seed meal. The best results of ingestion and digestibility coefficient of the components of the ration were obtained with the ration containing the boiled RSM. The processing treatments used in this work caused reduction at different rates in the level of anti-nutrients present in the raw seed of *Hevea brasiliensis* to tolerable level safe for consumption.

## DECLARATIONS

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### Authors' contribution

MA conceived the study, designed the study, collected data, contributed in data analysis, and writing the manuscript, coordinated the inputs of all the other authors; DFH performed critical reviewing of the manuscript and supervision of the study; FF, TA and TJ contributed in conception of the study and performed critical reviewing of the manuscript; CMA contributed in design of study, data collection and writing the manuscript; MM contributed in data analysis and performed critical reviewing of the manuscript; KMH contributed in conception of the study and critical reviewing of the manuscript.

### Competing interest

The authors declare they have no conflict of interest.

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