

# NUTRITIONAL COMPOSITION, *IN VITRO* GAS PRODUCTION AND *IN SACCO* DEGRADABILITY OF PROCESSED *Croton megalocarpus* NUTS FOR RUMINANT FEEDING

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<sup>✉</sup>Supporting Information

**ABSTRACT:** This study was conducted to evaluate the effects of processed croton nut on chemical composition, *in vitro* gas production and *in sacco* degradability. Four forms of croton nut namely: whole nut (WN), peeled nut (PN), De-husked nut (DhN) and De-fatted seed (DfS) were subjected to proximate analysis, Van Soest fibre fractionation, mineral composition analysis, phytochemical and aflatoxin tests. Degradability analyses were conducted using *in vitro* gas production and *in sacco* degradability techniques. Defatted seeds recorded significantly high level of CP and NFE (198 g/kg and 174 g/kg), whereas, ash content and ether extract (EE) were significantly high in WN (59 g/kg) and DhN (362 g/kg) respectively. Low fibre fractions of NDF (556 g/kg) and ADF (490 g/kg) were observed in DhN, while the mineral content was high in DfS which had calcium at 2.13 g/kg and phosphorus at 5.04 g/kg. High level of flavonoid was recorded in WN (124 g/kg), whereas low level of alkaloids was found in DfS (60 g/kg) and tannins in PN (7.1 g/kg). The potential *in vitro* gas production (a+b) was highest in DfS (22.2 ml/0.2 gDM) while potential *in sacco* degradability (a+b) was highest in DhN (58.4 %). High level of organic matter digestibility (OMD) (41 %) was observed in DfS. At  $k_p=0.025$  rumen outflow rate, DhN had the highest effective degradability of dry matter (56.6%), while the rate effective crude protein degradability was 80.0 %. Processing through peeling and dehushing improved the protein, energy and mineral content of DhN and DfS while crude fibre content reduced. Nutritional composition and degradability characteristics of all forms of croton nuts imply that they could be used in a total mixed ration (TMR) to supply requisite nutrients for maintenance of ruminant animals, while DhN and DfS could be used to supplement energy and protein for increased productivity.

**Keywords:** Chemical composition, Croton nut, degradability, Gas production technique, Processing.

**Abbreviations:** WN: whole nut; PN: peeled nut; DhN: dehusked nut; DfS: defatted seeds

## INTRODUCTION

The livestock sector accounts for 40% of agriculture's Gross Domestic Product (GDP) in developing countries and is not only a source of food and livelihood but enhances resilience against climate change extremities such as drought (Herrero et al., 2013; Nabarro and Wannous, 2014). The continuously growing human population as well as increased per capita income has led to increased demand for livestock-based products (Otte et al., 2019). Thus, livestock production ought to increase so as to meet the rising demand. Ruminant animals in tropical arid and semi-arid areas (ASAL) continue to play a key role of the rural households in developing countries where they are a major source of nourishment from products such as meat and milk as well as play social economic roles by providing income and acting as an economic safety net (Herrero et al., 2013).

Livestock production in the tropics is constrained by various factors which include inadequate nutrition, breeding and reproduction, disease and parasites among others (Kahi and Wasike, 2019). In confined systems, feeds account for up to 70% of the total cost of production (Makkar, 2014). Hence, variation in quantity and quality of feeds becomes a major constraint to livestock production. The problem of feed scarcity is further exacerbated by increased food – feed competition between human and livestock and decline in available land for feed production. Majority of ruminant animals (cattle, sheep and goats) in Kenya are reared in arid and semi-arid Counties (KNBS, 2019). In these areas, effects of climate change such as drought greatly reduces available feed resources consequently leading to low productivity and at time causing mortality of the animals (Makkar, 2014). Feeding strategies that optimise utilisation of available feed resources are thus critical to maintain ruminant productivity and preventing mortality.

Identification and introduction of alternative feed resources is a major avenue that could be used to mitigate feed scarcity. Locally available, low-cost feed resources could enhance resilience and adaptability of small holder farmers and pastoralists by allowing them to transit through adverse effects of climate change (Makkar, 2014). Evaluation of non-conventional feed resources for potential inclusion in mainstream livestock offers a preliminary step in determining the suitability of the identified feed resource before it can be included in livestock diets (Quansah and Makkar, 2012). One

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such feed with potential is croton nuts from *Croton megalocarpus* tree. Croton tree is adapted to different agro-ecological zones in the tropics and has multipurpose use such as provision of wood fuel, acting as a live fence and a source of bio fuel (Ndegwa et al., 2011). Croton tree produces up to 25 kg of nuts per year (Jacobson et al., 2018) which are reported to contain high CP content of up to 18%, crude fat (30%) and hence could be exploited for feeding livestock (Thijssen et al., 1996; Ndegwa et al., 2011). Farmers have been observed to collect and use croton nuts for feeding cows and goats during extreme dry seasons. However, there is limited information on the chemical composition, ant - nutritive factors and degradability of croton nuts. Moreover, there is also limited information on effects of processing various forms of croton such as peeling, dehiscing and oil extraction on the nutritive value for effective utilization of this underutilized feed resource. This study was therefore conducted to evaluate nutritional and phytochemical composition and ruminal degradation of the various processed forms of croton nut to facilitate its use in ruminant feeding.

## MATERIALS AND METHODS

### Site description

Samples of Croton nut were collected from Laikipia West and East sub counties of Laikipia County, which is located North - West of Mount Kenya at an altitude of between 1600 m and 2300 m above sea level with a total area of 9,700 km<sup>2</sup>. The area experiences a bimodal rainfall pattern with long rains between March and June and short rains between October and December separated by dry seasons (MoALF, 2017). The annual precipitation varies between 400 mm to 900 mm and average temperature is between 16 °C and 26 °C. The area lies in semi - humid, semi - arid, arid to very arid agro ecological zones IV - VII, and is considered arid and semi-arid (ASAL) (MoALF, 2017).

### Collection and processing of croton nuts

Mature croton nuts were collected from the ground, air dried under shade and processed into four forms which included whole nuts (WN), peeled nut (PN), dehusked nut (DhN) and defatted seeds (DfS). The whole nuts (WN) form comprised of unprocessed whole croton nuts with the outer peel (exocarp) and the hard woody husk (endocarp) intact. Peeled nuts (PN) consisted of nuts whose outer seed coat (peel/exocarp) was removed leaving the hard woody endocarp intact. De-husked nuts (DhN) consisted of the inner seeds after the removal of both the outer peel (exocarp) and the hard woody husk (endocarp). Defatted seeds (DfS) also referred to as Croton cake was the by-product of the seeds after oil extraction using a cold press. The DfS form was obtained from a commercial plant that extracts bio-diesel from croton in Laikipia County. After processing into various forms, the samples were then ground using a hummer mill to pass through a 2 mm screen and stored in air tight glass containers pending analyses.

### Chemical analyses

Ground samples of the various processed forms of croton were subjected to proximate analysis to determine dry matter (DM), ash, crude fibre (CF), ether extract (EE) and crude protein (CP) which were expressed on dry matter basis according to AOAC (1990). Nitrogen free extract (NFE) was calculated as the difference of the sum (%) of crude protein, crude fibre, ether extract and total ash from 100%. Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL), were sequentially determined using the method of Van Soest et al. (1991). Hemicellulose content was calculated as the difference between NDF and ADF, whereas cellulose was the difference between ADF and ADL. Gross energy (MJ/kg) was determined from 0.5 g of sample using a digital bomb calorimeter (CAL2K of Digital Data Systems (pty) ltd South Africa). Neutral detergent insoluble nitrogen and acid detergent insoluble nitrogen were determined from the residues of NDF and ADF using Kjeldahl method (AOAC, 1990). Nitrogen obtained was multiplied with a conversion factor (6.25) to obtain neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP).

Sodium (Na) and potassium (K) concentration was determined using atomic emission in a flame photometer while total available phosphorus (P) concentration was determined using Ultra Violet (UV) colorimeter (AOAC, 1990). Calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) concentrations were determined using an atomic absorption spectrophotometer (AAS) (AOAC, 1990).

### Phytochemicals and aflatoxin analyses

Flavonoids were extracted from the samples using organic solvents and expressed gravimetrically as outlined by Harbone (1984). Alkaloid determination was done by extraction from the samples using acetic acid dissolved in ethanol (Harbone, 1984). Tannin content was determined using Folin-Coicalteu reagent and determination of absorbance was done at 725 nm using a UV-visible spectrophotometer (AOAC, 1990). Aflatoxins were extracted using methanol and levels determined by ELISA testing kit. The amount of aflatoxin was expressed in parts per billion (ppb) calculated from the standard aflatoxin curve (Leszczyńska et al., 2001).

### In vitro gas production

In vitro gas procedure was conducted following the procedure of Menke and Steingass (1988). Rumen liquor was drawn in the morning from two mature fistulated Friesian steers. The steers had 450±25 kg live weight and were fed on Rhodes grass (*Chloris gayana*) hay and wheat bran at 90% and 10% respectively of the total ration at 3% of their body

weight at 08:00 hours and 17:00 hours for maintenance purposes. Mineral licks and water were provided ad libitum. This was done so as to maintain a stable rumen environment before the rumen liquor was collected. Collected rumen liquor was strained through four layers of cheese cloth into a pre-warmed, vacuum flask and kept at 39°C under CO<sub>2</sub> atmosphere. About 0.2g of 1mm ground samples (WN, PN, DhN and DfS) were weighed into the glass syringes. A mixture of 30ml of rumen liquor and buffer in the ratio of 1:2 was added into each of the 100ml calibrated glass syringes that were pre-warmed to 39°C. Oil was applied to the pistons to facilitate ease of movement and prevent gas escape. Two blank syringes with rumen liquor without a feed sample were included as controls. All syringes were incubated in a water bath maintained at 39°C and shook periodically. Gas production readings were recorded at 0 and after 3, 9, 12, 24, 48, 72 and 96 hours of incubation.

The gas production characteristics were computed by fitting the mean gas volumes to the exponential equation of Ørskov and McDonald (1979) using Neway Excel Computer program (Chen X. B., Rowett Research Institute, Aberdeen UK).

$$Y = a + b(1 - e^{-ct}) \quad (\text{Ørskov and McDonald, 1979}) \quad (1)$$

Where:  $Y$  is gas production (ml/0.2g) at time  $t$ ,  $a$  is gas production (ml) from immediately soluble fraction,  $b$  is gas production (ml) from insoluble fraction,  $a+b$  is gas production from potential degradable fraction,  $c$  is the rate constant of gas production per hour (h),  $t$  is the incubation time in hours and  $e$  is the exponential constant (2.718).

*In vitro* gas production parameters were used to estimate organic matter digestibility (OMD), metabolisable energy (ME), Dry Matter intake (DMI) and short chain fatty acids (SCFA) using the models presented in Equations 2 to 5.

$$OMD(\%) = 14.88 + 0.889 GV + 0.45 CP + 0.0651 XA \quad (\text{Menke and Steingass, 1988}) \quad (2)$$

$$ME(\text{MJ/Kg}) = 2.20 + 0.136 GV + 0.057 CP \quad (\text{Makkar and Becker, 1996}) \quad (3)$$

$$DMI(\text{kg/day}) = 1.66 + 0.49a + 0.0297b - 4c \quad (\text{Blümmel and Ørskov, 1993}) \quad (4)$$

$$SCFA(\text{mmol/L}) = 0.0222 GV - 0.00425 \quad (\text{Makkar, 2005}) \quad (5)$$

Where:  $GV$  is gas production after 24 hours,  $CP$  is crude protein and  $XA$  ash content of the processed form of croton,  $a$ ,  $b$  and  $c$  are constants as described in Equation 1.

#### ***In sacco* degradation (nylon bag technique)**

*In sacco* degradation of the various forms of croton was carried out using Nylon bag technique as described by Ørskov (2000). Two mature fistulated Friesian steers weighing  $450 \pm 25$  kg live weight were used. The steers were fed on Rhodes grass (*Chloris gayana*) hay and wheat bran at 90% and 10% respectively of the total ration at 3% of their body weight at 08:00 hours and 17:00 hours for maintenance purposes. Mineral licks and water were provided ad libitum. This was done so as to maintain a stable rumen environment. Five grams of each processed sample of croton was weighed into duplicate nylon bags (12cm by 6cm, 50µm pore size). The bags were incubated for 0, 9, 12, 16, 24, 48 and 72 hours in the rumen. Zero-hour washing was measured by soaking nylon bags containing the sample in water maintained at 39 °C for 1 hour. Bags from zero hour washing and those retrieved from the rumen were washed thoroughly under running cold water for 15 minutes until the washing water was clear. The bags with the residue were then dried at 60°C for 48 hours in a forced air oven and dry matter loss determined as the difference from the original weight. Crude protein and neutral detergent fibre (NDF) from the residue were then analysed. The DM, CP and NDF degradability characteristics were determined by fitting the degradability data to the exponential Equation 6 of Ørskov and McDonald (1979) using Neway Excel Computer program (Chen X. B., Rowett Research Institute, Aberdeen UK).

$$P = a + b(1 - e^{-ct}) \quad (\text{Ørskov and McDonald, 1979}) \quad (6)$$

Where:  $P$  is the degradability of (DM, CP and NDF) incubated in the rumen at time  $t$  in hours,  $a$  is the percentage of rapidly soluble fraction,  $b$  is the percentage of insoluble but fermentable fraction,  $a+b$  is potential percentage of degradability,  $c$  is the rate of constant degradation per hour ( $\text{h}^{-1}$ ) and  $e$  is the exponential constant (2.718).

Effective degradability (ED) of DM, CP and NDF was calculated using Equation (7).

$$ED = a + b\left(\frac{c}{c+kp}\right) \quad (\text{McDonald, 1981}) \quad (7)$$

Where:  $a+b$  is the potential degradability,  $c$  is the rate constant degradability per hour (h),  $kp$  is the ruminal outflow rate. The following outflow rates ( $kp$ ) per hour were considered (0.025, 0.05 and 0.08). Rumen undegradable protein (RUP) was calculated by subtracting effective degradable CP% from 100%. The DM index value (IV) which denotes the fraction of the feed that would provide nutrients to the animal for its maintenance needs was calculated using Equation 8. A feed with an index value above 33 would provide sufficient nutrients to the animal for its maintenance needs.

$$IV = a + 0.4b + 200c \quad (\text{Ørskov and Shand (1997)}) \quad (8)$$

Where:  $a$ ,  $b$  and  $c$  are as described in Equation 6.

#### **Statistical analysis**

Analysis of variance (ANOVA) was carried out on proximate composition, fibre fractions, minerals composition, gross energy (GE) and phytochemicals as well as *in vitro* gas production and *in sacco* degradability parameters. The analysis was based on completely randomized design using STATA (2017). Significant differences between the means were tested using Tukey's honest significance difference (THSD). The following statistical model was used

$$y_{ij} = \mu + f_i + e_{ij} \quad (9)$$

Where:  $y_{ij}$  = chemical composition, *in vitro* gas production and *in sacco* degradability parameters,  $\mu$  = mean of the different forms of *Croton megalocarpus*,  $f_i$  = forms of croton nuts ( $i$ = WN, PN, DhN and DfS),  $e_{ij}$  = error term.

### Ethical approval

All process of *in vivo* study was in according to animal welfare rules and approved by university ethical committee.

## RESULTS

### Proximate composition

Proximate composition of the various forms of croton nut is presented in Table 1. Peeled nut had significantly high DM content while WN did not differ significantly from DhN and DfS ( $P < 0.05$ ). Defatted seeds had significantly high CP content compared to other forms while the lowest level of CP was recorded in WN and PN which were not significantly different ( $P < 0.05$ ). The CF content was significantly low in DhN compared to the other forms while the ash content was significantly high in WN followed by DfS, but no significant difference was observed between PN and DhN. The EE content did not differ significantly between WN and PN but was significantly high in DhN at 363g/kg and significantly low in DfS (113g/kg;  $P < 0.05$ ). The NFE in all forms did not differ significantly. Gross energy was highest in the DhN (21.1MJ/kg) and lowest in PN (17.3MJ/Kg) although the differences were not significant.

### Fibre composition

Fibre composition of the various forms of croton nut is presented in Table 2. Processing by dehusking and defatting resulted to lower NDF content in DhN and DfS to 576 g/kg and 556g/kg respectively, compared to WN and PN forms ( $P < 0.05$ ). Hemicellulose content was highest in PN (205 g/kg) ( $P < 0.05$ ). The cellulose level ranged between 94g/kg in DhN to 181g/kg in WN. The NDICP ranged between 16 in PN to 24 in DfS while ADICP ranged between 16 to 21 in both WN and DfS ( $P > 0.05$ ). There were no significant differences in ADF, ADL, cellulose, NDICP and ADICP among the croton forms ( $P > 0.05$ ).

**Table 1 - Proximate composition of the various forms of croton nut (g/kg)**

Nutritional parameter	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
Dry matter	893 <sup>a</sup>	963 <sup>b</sup>	917 <sup>c</sup>	919 <sup>c</sup>	0.763	$P < 0.001$
Crude protein	89 <sup>a</sup>	80 <sup>a</sup>	158 <sup>b</sup>	198 <sup>c</sup>	1.487	$P < 0.001$
Crude fibre	522 <sup>a</sup>	579 <sup>b</sup>	336 <sup>c</sup>	476 <sup>a</sup>	2.747	$P < 0.001$
Ether extract	185 <sup>a</sup>	175 <sup>a</sup>	363 <sup>b</sup>	113 <sup>c</sup>	2.824	$P < 0.001$
Ash	59 <sup>a</sup>	23 <sup>b</sup>	24 <sup>b</sup>	38 <sup>c</sup>	0.449	$P < 0.001$
Nitrogen free extract	143 <sup>a</sup>	143 <sup>a</sup>	120 <sup>a</sup>	174 <sup>a</sup>	0.847	$P < 0.001$
Gross energy (MJ/kg)	18.1	17.3	21.1	19.3	0.826	NS

<sup>a,b,c</sup>: Means in the same row without common letter are different at  $P < 0.05$ ; SEM = standard error of the mean; Prob.= probability; NS = not significant.

**Table 2 - Fibre composition of the various forms of croton nut (g/kg)**

Fibre components	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
NDF	686 <sup>a</sup>	741 <sup>a</sup>	576 <sup>b</sup>	556 <sup>b</sup>	2.36	$P < 0.001$
ADF	506	536	482	490	1.10	0.357
ADL	341	377	392	367	1.62	0.781
Hemicellulose	180 <sup>ab</sup>	205 <sup>b</sup>	94 <sup>ac</sup>	66 <sup>c</sup>	2.02	$P < 0.010$
Cellulose	181	168	94	132	1.39	0.089
NDICP	22	16	23	24	0.159	0.155
ADICP	21	16	17	21	0.115	0.063

<sup>a,b,c</sup>: Means in the same row without common letter are different at  $P < 0.05$ ; NDF = neutral detergent fibre; ADF = acid detergent fibre; ADL = acid detergent lignin; NDICP = neutral detergent insoluble crude protein; ADICP = acid detergent insoluble crude protein; SEM = standard error of the mean; Prob. = probability.

### Minerals composition

Mineral content of the various forms of croton nut is presented in Table 3. Processing by defatting enhanced the macro minerals (Ca, P, Mg and Na) in DfS ( $P < 0.05$ ). Whole nut recorded the highest level of potassium (14.27 g/Kg)

( $P < 0.05$ ). Amongst the micro minerals, Fe was highest in WN (0.113 g/kg), Mn in DhN (0.047g/kg) and Zn in DfS (0.049 g/kg) at  $P < 0.05$  compared to the other forms.

**Table 3 - Mineral composition of the various forms of croton nut (g/kg)**

Mineral composition	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
<b>Macro minerals</b>						
Calcium	1.51 <sup>a</sup>	1.69 <sup>ab</sup>	1.82 <sup>ab</sup>	2.13 <sup>b</sup>	0.084	0.0324
Phosphorus	3.21 <sup>ab</sup>	2.78 <sup>a</sup>	4.21 <sup>bc</sup>	5.04 <sup>c</sup>	0.284	<0.001
Magnesium	0.46 <sup>ab</sup>	0.35 <sup>a</sup>	0.57 <sup>bc</sup>	0.71 <sup>c</sup>	0.042	<0.001
Sodium	0.79 <sup>a</sup>	0.14 <sup>b</sup>	0.34 <sup>c</sup>	2.27 <sup>d</sup>	0.251	<0.001
Potassium	14.27 <sup>a</sup>	4.36 <sup>b</sup>	3.66 <sup>b</sup>	5.41 <sup>c</sup>	1.294	<0.001
<b>Micro minerals</b>						
Iron	0.113 <sup>a</sup>	0.051 <sup>b</sup>	0.063 <sup>bc</sup>	0.075 <sup>c</sup>	0.0071	<0.001
Manganese	0.024 <sup>a</sup>	0.029 <sup>a</sup>	0.047 <sup>b</sup>	0.046 <sup>b</sup>	0.0031	<0.001
Zinc	0.022 <sup>ab</sup>	0.017 <sup>a</sup>	0.034 <sup>bc</sup>	0.049 <sup>c</sup>	0.004	0.001
Copper	0.007	0.015	0.019	0.008	0.0027	0.401

<sup>a,b,c,d</sup>; Means in the same row without common letter are different at  $P < 0.05$ ; SEM = standard error of the mean; Prob. = probability.

### Phytochemicals and aflatoxin content

Phytochemical composition and aflatoxin levels of the various forms of croton nut is presented in Table 4. Flavonoid content in WN was significantly higher (124 g/kg) ( $P < 0.05$ ) from other forms. Alkaloids ranged from 60g/kg in DfS to 69g/kg in WN ( $P > 0.05$ ). Both WN and DfS had the highest tannin level (9.6 g/kg) ( $P < 0.05$ ). Aflatoxin level was highest in DhN (21.1 ppb) and least in PN (6.4 ppb).

### In vitro gas production

*In vitro* gas production fermentation characteristics of the various forms of croton nut are presented in Table 5. There was no difference in gas production from the readily soluble fraction (a) among the forms ( $P > 0.05$ ). However, highest gas production of (b) and (a+b) were observed in DfS at (18.6 ml) and (22.2 ml) respectively ( $P < 0.05$ ). Defatted seeds recorded the highest OMD (41.0%), ME (5.9 MJ/kg), and SCFA (0.419 mmol/L) while PN had the least OMD (29.8%), ME (4.3 MJ/kg) and SCFA (0.271 mmol/L).

### In sacco DM degradability

*In sacco* DM degradability characteristics of the various forms of croton nut are presented in Table 6. Dehusked nut had highest rapidly soluble DM fraction (a ~ 42.8%) and potentially degradable DM fraction (a+b ~ 58.4%) ( $P < 0.05$ ), with the rate constant of degradation (c) ranging between 0.02 in WN to 0.2 in DhN. Effective dry matter degradability (EDDM) among the various forms was observed to reduce as the rumen outflow rate increased. Dehusked nut (DhN) had consistently higher percentages of EDDM and at all rumen outflow rates and a converse trend was true for PN. Dehusked nut also recorded the highest IV 90.1 ( $P < 0.05$ ).

### In sacco CP degradability

*In sacco* CP degradability characteristics of the various forms of croton nut are presented in Table 7. Rapidly degradable fraction of protein (a) was highest in WN (4.1%) ( $P < 0.05$ ). At  $p < 0.05$ , slowly degradable fraction (b) and potential degradable fraction (a+b) were highest in DhN (87.8%) and (87.9%) and lowest in WN (59.4%) and (63.5%) respectively. The rate constant of degradability per hour (c) was highest in PN (0.26) and lowest in DhN (0.02) whereas highest rumen undegradable protein was recorded in WN (36.4%) and the lowest recorded in DhN (12.0%). At  $kp = 0.025$ , effective degradable crude protein for DhN (80.0%) and DfS (65.1%) were different ( $P < 0.05$ ) from that of WN and PN. The rumen undegradable protein (RUP) among all forms of croton nuts at 0.025  $kp$  was low compared to RUP at 0.05 $kp$  and 0.08 $kp$  ( $P < 0.05$ ).

### In sacco NDF degradability

The NDF degradability characteristics of the various forms of croton nut are presented in Table 8. Significant difference in NDF degradability was observed in rapidly degradable fraction (a) which was highest in DhN (17.2%) compared to the other forms of croton nut ( $P < 0.05$ ). There was no significant difference among the various forms of croton nut for b, a+b, and c. At 0.025 $kp$  and 0.08 $kp$ , effective degradability NDF was significantly high in DhN compared to the other forms. However, at 0.05 $kp$  there was no significance difference among all forms of croton nut in EDNDF.

**Table 4 - Phytochemical and aflatoxin content of the various forms of croton nut (g/kg).**

Anti-nutritive factors	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
Flavonoids	124 <sup>a</sup>	57 <sup>b</sup>	43 <sup>b</sup>	64 <sup>b</sup>	1.01	P<0.0014
Alkaloids	69	67	62	60	1.85	0.307
Tannins	9.6 <sup>a</sup>	7.1 <sup>b</sup>	8.9 <sup>ab</sup>	9.6 <sup>a</sup>	0.04	P<0.021
Aflatoxin (ppb)	14	6.4	21.1	9.9	3.13	ND

<sup>a,b,c</sup>: Means in the same row without common letter are different at (P<0.05); SEM = standard error of the mean; Prob. = probability; ND = not determined.

**Table 5 - *In vitro* gas production of the various forms of croton nut (ml gas/0.2g dry matter).**

Gas production parameters	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
<i>a</i>	1.4	4.2	2.4	3.7	0.465	P<0.083
<i>b</i>	18.6 <sup>a</sup>	10.6 <sup>b</sup>	14.3 <sup>ab</sup>	18.4 <sup>a</sup>	1.28	P<0.004
<i>a+b</i>	20.1 <sup>ab</sup>	14.9 <sup>a</sup>	16.8 <sup>ab</sup>	22.2 <sup>b</sup>	1.13	P<0.019
<i>c</i>	0.08	0.06	0.10	0.08	0.007	P<0.096
OMD	34.1 <sup>ab</sup>	29.8 <sup>a</sup>	35.3 <sup>b</sup>	41.0 <sup>c</sup>	1.54	P<0.004
ME (MJ/Kg)	5.0 <sup>a</sup>	4.3 <sup>a</sup>	5.1 <sup>a</sup>	5.9 <sup>b</sup>	0.218	P<0.005
DMI (kg/day)	2.6	3.8	2.8	3.6	0.226	P<0.103
SCFA (mmol/L)	0.37 <sup>a</sup>	0.27 <sup>ab</sup>	0.32 <sup>ab</sup>	0.41 <sup>b</sup>	0.022	P<0.035

<sup>a,b,c</sup>: Means in the same row without common letter are different at P<0.05; *a* = gas production (ml) from immediately soluble fraction; *b* = gas production (ml) from insoluble fraction, *a+b* = potential gas production (ml); *c* = the rate constant of gas production per hour; OMD = organic matter digestibility; ME = metabolisable energy; DMI = dry matter intake; SCFA = short chain fatty acids; SEM = standard error of the mean; Prob. = probability.

**Table 6 - *In sacco* DM degradability characteristics for various forms of croton nut (%).**

DM degradability parameters	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob
<i>a</i>	29.5 <sup>a</sup>	26.4 <sup>b</sup>	42.8 <sup>c</sup>	33.4 <sup>d</sup>	2.33	P<0.001
<i>b</i>	18.8 <sup>ab</sup>	22.5 <sup>b</sup>	15.6 <sup>ab</sup>	12.5 <sup>a</sup>	1.49	P<0.028
<i>a+b</i>	48.3 <sup>a</sup>	49.0 <sup>a</sup>	58.4 <sup>b</sup>	46.0 <sup>a</sup>	1.87	P<0.011
<i>c</i>	0.02	0.01	0.20	0.05	0.032	P<0.051
EDDM ( <i>k<sub>p</sub></i> =0.025)	37.8 <sup>a</sup>	30.7 <sup>b</sup>	56.6 <sup>c</sup>	41.8 <sup>d</sup>	3.56	P<0.001
EDDM ( <i>k<sub>p</sub></i> =0.05)	34.9 <sup>a</sup>	28.8 <sup>b</sup>	55.1 <sup>c</sup>	39.7 <sup>d</sup>	3.68	P<0.001
EDDM ( <i>k<sub>p</sub></i> =0.08)	33.2 <sup>a</sup>	28.0 <sup>b</sup>	53.8 <sup>c</sup>	38.3 <sup>d</sup>	3.64	P<0.001
IV	41.0 <sup>a</sup>	36.6 <sup>a</sup>	90.1 <sup>b</sup>	48.5 <sup>a</sup>	8.44	P<0.017

<sup>a,b,c,d</sup>: Means in the same row without common letter are different at P<0.05; *a* = is the rapidly soluble fraction; *b* = is the insoluble but fermentable fraction; *a+b* = is the potentially degradable fraction; *c* = is the rate constant of degradation; IV = index value; EDDM = effective degradability of dry matter; *k<sub>p</sub>* = rumen outflow rate; SEM = standard error of the mean; Prob. = probability.

**Table 7 - *In sacco* CP degradability characteristics of the various forms of croton nut (%).**

CP degradability	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
<i>a</i>	4.1 <sup>a</sup>	0.02 <sup>b</sup>	0.08 <sup>b</sup>	0.4 <sup>b</sup>	0.660	P<0.002
<i>b</i>	59.4 <sup>a</sup>	75.0 <sup>b</sup>	87.8 <sup>c</sup>	74.7 <sup>b</sup>	3.81	P<0.001
<i>a+b</i>	63.5 <sup>a</sup>	75.0 <sup>b</sup>	87.9 <sup>c</sup>	75.1 <sup>b</sup>	3.26	P<0.001
<i>c</i>	0.06 <sup>a</sup>	0.26 <sup>b</sup>	0.02 <sup>b</sup>	0.16 <sup>c</sup>	0.0312	P<0.001
EDCP ( <i>k<sub>p</sub></i> =0.025)	46.2 <sup>a</sup>	68.5 <sup>a</sup>	80.0 <sup>b</sup>	65.1 <sup>b</sup>	4.59	P<0.001
EDCP ( <i>k<sub>p</sub></i> =0.05)	36.8 <sup>a</sup>	63.0 <sup>b</sup>	73.5 <sup>c</sup>	57.5 <sup>d</sup>	5.06	P<0.001
EDCP ( <i>k<sub>p</sub></i> =0.08)	29.8 <sup>a</sup>	57.4 <sup>b</sup>	66.9 <sup>c</sup>	50.4 <sup>d</sup>	5.16	P<0.001
RUP ( <i>k<sub>p</sub></i> =0.025)	53.7 <sup>a</sup>	31.4 <sup>a</sup>	19.9 <sup>b</sup>	34.8 <sup>b</sup>	4.59	P<0.001
RUP ( <i>k<sub>p</sub></i> =0.05)	63.1 <sup>a</sup>	36.9 <sup>b</sup>	26.4 <sup>c</sup>	42.4 <sup>d</sup>	5.06	P<0.001
RUP ( <i>k<sub>p</sub></i> =0.08)	70.1 <sup>a</sup>	42.5 <sup>b</sup>	33.0 <sup>c</sup>	49.5 <sup>d</sup>	5.16	P<0.001

<sup>a,b,c,d</sup>: Means in the same row without common letter are different at P<0.05; *a* = is the rapidly soluble fraction; *b* = is the insoluble but fermentable fraction; *a+b* = is the potentially degradable fraction; *c* = is the rate constant of degradation; EDCP = effective degradability of crude protein; *k<sub>p</sub>* = rumen outflow rate; RUP = rumen undegradable protein; SEM = standard error of the mean; Prob. = probability.

**Table 8 - In sacco NDF degradability characteristics of the various forms of croton nuts (%)**

NDF degradability	Whole nut (WN)	Peeled nut (PN)	Dehusked nut (DhN)	Defatted seeds (DfS)	SEM	Prob.
A	6.5 <sup>a</sup>	2.0 <sup>a</sup>	17.2 <sup>b</sup>	1.6 <sup>a</sup>	2.41	0.002
B	15.1	9.4	10.1	15.8	1.28	0.144
a+b	21.6	11.4	27.3	17.4	2.44	0.065
C	0.03	0.01	0.33	0.35	0.0052	0.714
EDNDF ( $k_p=0.025$ )	15.0 <sup>a</sup>	5.9 <sup>b</sup>	22.9 <sup>c</sup>	9.1 <sup>b</sup>	2.47	P<0.001
EDNDF ( $k_p=0.05$ )	11.2	7.5	21.2	6.8	2.45	0.053
EDNDF ( $k_p=0.08$ )	10.2 <sup>a</sup>	3.7 <sup>b</sup>	20.1 <sup>c</sup>	5.5 <sup>b</sup>	2.43	P<0.001

<sup>a,b,c,d</sup>: Means in the same row without common letter are different at P<0.05; a = is the rapidly soluble fraction; b = is the insoluble but fermentable fraction; a+b = is the potentially degradable fraction; c = is the rate constant of degradation; EDNDF = effective degradability of neutral detergent fibre;  $k_p$  = rumen outflow rate; SEM = standard error of the mean; Prob. = probability.

## DISCUSSION

### Nutritional composition

The DM content in all forms was above 86%, which is the recommended level for storage of feeds. Conversely, this implied low moisture content that is critical in preventing growth of fungi and reducing aflatoxin contamination (Mahato et al., 2019). The high moisture content in WN suggests that the peel acts as a barrier against loss and itself contains moisture. Whole nuts and DfS had high ash contents indicating that they could be good sources of minerals for grazing animals during the dry seasons hence averting the effects of mineral deficiencies such as impaired growth, poor health and reduced reproductive performance in ruminants (Lengarite et al., 2012). This is corroborated by mineral results whereby, Ca and P levels of all forms of croton nut in this study were within the recommended critical maintenance level (1.2 - 2.6g/kg Ca) and (1.4g/kg P) respectively for ruminant animals (ARC, 1980). The K level in WN was above 8g/kg even though, the Mg level in all forms was below (2g/kg) recommended level for grazing animals, (Mirzaei, 2012). The level of Fe was above the recommended level (0.05g/kg) for grazing animals (ARC, 1980). Both DhN and DfS contained the recommended critical level of Zn (0.03g/kg) which is sufficient for cattle, sheep and goats (ARC, 1980).

Removal of the husks and defatting effectively elevated CP content as reflected in DhN and DfS forms. The CP in all croton forms was above the recommended (80g/kg) required for maintenance in grazing ruminant animals (NRC, 2001). Moreover, DhN and DfS CP levels were within 140g/kg to 165g/kg recommended for growth and increased milk production in lactating animals (NRC, 2001). Defatting reduced the EE content considerably in DfS making it suitable for storage by reducing the amount of oils which when oxidised cause rancidity hence feed spoilage.

Removal of the outer peel and husks (hard woody endocarp encasing the seeds) lowered the fibre levels considerably in DhN and DfS. Neutral detergent fibre level in these forms was between 450 g/kg to 650 g/kg. These forms may be classified as medium quality feed, a predominant characteristic of tropical feed stuffs (Singh and Oosting, 1992). Feeds in this category can achieve the required gut health of ruminant animals by enhancing optimum feed intake, stimulating rumen function and increasing chewing of cud (Singh and Oosting, 1992). Moderate crude protein levels (80 – 90 g/kg) in WN and PN could play a fundamental role in mitigating lowered fibre digestibility that may be occasioned by the high NDF through availing of rumen ammonia nitrogen necessary for optimal functioning of the rumen ecosystem (Van Soest, 1994). There was no difference in NDICP among all forms of croton nut an indication that the degradability of insoluble-protein fraction was similar in all forms. NDICP represent the insoluble fraction of protein that remains after extraction with neutral detergent solution and is usually assumed to be insoluble (Mustafa et al., 2001). This fraction is a measure of nitrogen availability and constitutes a major portion of ruminal undegradable protein content (Mustafa et al., 2001).

High flavonoid content in all forms of croton nuts is an indication that croton nut could be included in ruminant feed rations to confer improved growth performance, health and improved rumen fermentation (Panche et al., 2016). A study by Kong et al. (2019) showed that flavonoid supplementation improved the average daily gain by alleviating stress during weaning of Holstein calves. Low level of tannins (<50 g/kg) similar to those recorded in this study could confer beneficial effects to ruminant animals such as reduction in ruminal protein degradation thus availing essential amino acids for absorption in the small intestines (Frutos et al., 2004). The level of aflatoxin observed in this study was within the minimum recommended level of 20ppb for complete and complementary feed materials used for feeding cattle, sheep and goats except for DhN (Kotinagu et al., 2015). The high level of aflatoxin in DhN could be attributed to high level of oil which provides conducive environment for growth of fungi resulting in production of aflatoxins (Filazi and Sireh, 2013). Therefore, proper handling and storage of DhN is crucial to prevent conditions that could encourage growth of fungi.

### In vitro gas production

Amount of gas produced in *in vitro* gas digestibility method is an indicator of the rate and extent of feed digestion (Makkar, 2005). Gas production is affected by the composition, bioavailability of nutrients and presence of anti-nutritive factors in a feed. The higher levels of gas production observed in DfS compared to other forms of croton could be

attributed to high levels of fermentable carbohydrates and protein which produce more gas when acted upon by rumen microbes (Makkar, 2005). Quality of roughage in a feed determines the nutritive value that the feed would confer when fed to an animal. The presence of high amount of fibre in a feed increases the rumen pool of indigestible fibre lignin which impedes the action of fibrolytic microbes that act on cellulose and hemicellulose (Venkateswarlu et al., 2013). This consequently reduces fermentable fibre as observed in PN.

Observed reduced fermentation characteristics in DhN could be attributed to high levels of EE in this form. Although the type of fat was not differentiated in present study, presence of poly unsaturated fatty acids (PUFA) has been shown to reduce activity of fibre degrading microbes resulting to lower degradation and low gas production as observed in this study (Maia et al., 2010). It has been shown that excess oil of the long fatty acids in a feed (more than 3 - 5%) of the dry matter has a toxic effect on ruminal microorganisms especially bacteria which form the major fibrolytic colonies (Castillo-González et al., 2014). High predicted OMD and DMI in DfS implied better nutritive value in this form indicating that ruminant animals could consume higher amounts compared to the other forms (Negesse et al., 2016). The markedly high level of SCFA produced by DfS indicated that this form was better placed to supply the ruminant animals with the requisite energy to support production.

#### ***In sacco* degradability**

High dry matter degradability of rapidly degradable fraction (a) in DhN is an indication of high soluble nutrients which could be combined with low quality roughages to provide protein and energy needed by microbes. Slowly degradable fraction (b) of DM in all forms of croton nut was low compared to rapidly degradable fraction. Low fibre quality limit the ability of microbes in effectively degrading the feed by making it difficult for rumen microorganism to attach on the feed particles (Venkateswarlu et al., 2013). The dry matter rate constant of degradation (c) was comparable to various conventional feed resources such as coconut meal, peanut meal and whole cotton seeds (0.2-0.05 per hour) (Chumpawadee et al., 2005). This rate is important as it determines rumen fill and exerts direct effect on intake (Chumpawadee et al., 2005). At rumen outflow rate of  $kp=0.05$ , effective degradability (DM) of various forms of croton in this study were within the range (24.3 – 60.9%) observed for conventional protein sources which include soy bean meal, whole cotton seed, coconut meal and fish meal (Chumpawadee et al., 2005). This fraction represents the total amount of nutrients which can be captured by rumen microbes for their growth, production of VFAs and synthesis of microbial protein (Lanyasunya et al., 2006). The IV of all croton forms in this study were within the acceptable level of >33 as recommended by (Ørskov and Shand 1997). This level indicates sufficient nutrients that an animal needs to consume to meet its daily maintenance needs.

The low level of rapidly soluble fraction of CP (a) observed in this study is within the recommended <40% for effective degraded protein (Lanyasunya et al., 2006). At this level, the (a) fraction does not overwhelm rumen microbes through production of excess nitrogen in form of ammonia, thus, maintaining an optimal protein-energy balance. Feeds with high slowly degradable fraction (b) avail required nitrogen in small amounts which are effectively utilized by rumen microbes. Effective degradability of crude protein provides an estimate of the total amount of protein captured by the rumen microbes for growth and synthesis of microbial protein (Lanyasunya et al., 2006). This fraction was high in DhN an indication that a considerable amount of protein in this form was degraded in the rumen. The remaining amount of protein regarded as rumen undegradable protein (RUP) represents the fraction of protein that is not degraded in the rumen and is termed as rumen by pass protein (Gao et al., 2015). Rumen by pass protein is available at the lower gut (small intestines) where combined with microbial protein contribute to protein requirements of the animal for maintenance and production. In this study WN was a good source of RUP and could be used to provide this form of protein in ruminant diets.

## **CONCLUSION**

Processing through dehusking and defatting had the most significant impact on the nutritional composition of croton nuts. The two methods improved the nutritional profiles of protein, energy and mineral contents while reducing the fibre fractions compared to where peeling or no-processing was done. Degradability of dehusked and defatted forms of croton nuts was also high compared to the peeled and unprocessed whole nut forms. However, nutritional value of all forms of croton nuts was adequate and could be used in a total mixed ration (TMR) for maintenance purposes. In particular, dehusked and defatted forms have potential utilisation as protein supplements which could additionally supply energy and minerals for increased ruminant productivity on low quality basal diets. Microbial, enzymatic or chemical pre-treatment of the WN and PN forms prior to feeding could be explored to improve any observed lowered feed digestibility. Further studies to assess the effect of feeding croton on palatability, level of intake and production performance of ruminants are required.

## **DECLARATIONS**

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## Authors' contribution

All authors contributed equally to this work.

## Conflict of interests

The authors declare that there are no competing interests.

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