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Effect of Feeding Rubber (*Hevea brasiliensis*) Seed Meal Based Diets on Intake, Nutrient Digestibility and Growth Performance in Goats

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ABSTRACT

The study investigated the processed rubber seed meal (RSM) as an alternative feed ingredient in goat kids. The chemical analysis indicated that RSM contained 30.85 % CP and 14.13 MJ ME / kg DM. The methods employed to detoxify raw RSM were soaking, boiling with and without decanting and fermentation for different time periods and the residual hydrogen cyanide levels were below the detectable levels in all processed RSM. The *in vitro* true digestibility (%) of raw and boiled RSM with respect to DM, OM and NDF was 92.11, 92.47 & 63.48 and 87.88, 88.38 & 59.64, respectively. For feeding trial, 15 kids of comparable body weight and age were distributed into three groups of 5 each in completely randomized design. The compounded feed mixture (CFM) containing groundnut cake (GNC) as a protein supplement was served as control (T1) and test CFMs contained boiled RSM replacing 12.5 % (T2) and 25 % (T3) of GNC nitrogen in control. Each CFM was fed to one of the three groups while the COFS-29 hay was offered *ad libitum* for a period of fourteen weeks. The total dry matter intake (DMI) in terms of g/d (273.4 to 307.9) or % BW (2.68 to 2.94) was similar among the groups. However, the DMI in terms of $g/kgW^{0.75}$ was lower ($P<0.05$) in T3 (47.82) compared to control (52.72) which might be due to lower hay intake in T3 (57.66 g/d) compared to T1 (81.12 g/d). The average daily gain [22.75 (T3) to 32.24 g (T1)] and feed conversion ratio [3.85 (T2) to 4.07 (T3)] were non-significant among the treatments. The digestibility trial conducted during terminal week revealed that the apparent digestibility (%) of DM, OM, CP, EE, NDF and ADF were comparable among treatment diets. The digestible OM in DM was non-significantly ranged between 74.58 (T2) and 71.35 % (T1). It was concluded that boiled RSM can replace conventional protein source upto 25% on nitrogen equivalent basis in CFM of goat kids for sustainable small ruminant production.

KEYWORDS: Detoxification, Digestibility, Goat kids, Growth, Intake, Rubber seed meal

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INTRODUCTION

Goats play a crucial role in the livelihood of small and marginal farmers in India, particularly in ecologically fragile regions like arid, semi-arid, and hilly areas. India ranks second in the global goat population with 148.88 million goats (20th Livestock Census, 2019). Feed accounts for about 75% of livestock production costs, and there is a current net shortage of 44% of concentrate feed ingredients, 10.95% of dry crop leftovers, and 35.6% of green fodder (ICAR-IGFRI, Vision 2050). The rise in both human and livestock population has led to food insecurity, with a high demand for conventional feed ingredients with escalating prices. This necessitates the identification of alternative, nutrient-rich, and cost-effective feedstuffs. Rubber seed meal (RSM) offers a promising alternative and rubber seed trees are predominantly found in Southeast Asia and West Africa (Lukman et al., 2018). RSM contains 30.68% crude protein, 22.27% crude fat, and 33.53% nitrogen-free extract (Aguihe et al., 2017). Rubber seed has high protein content and also has numerous anti-nutritional factors such as hydrogen cyanide (HCN), phytic acid, oxalic acid, tannin, saponin and trypsin inhibitor. Among these, cyanogen compounds are more prominent and HCN content in raw RSM can lead to adverse effects such as growth depression, decreased protein synthesis, and immune system suppression (Ukpebor et al., 2007; Deng et al., 2015). This necessitates detoxification processes such as heat treatment, storage, enzymatic reaction, and fermentation for inclusion of RSM in animal diets.

Udo et al. (2021) reported that the diet incorporated with 10% boiled RSM was better utilized by West African dwarf bucks and could be used to formulate alternative concentrate for goats. However, the available literature is not sufficient to recommend the safe inclusion level of RSM in ruminant diets. Given limited research on using detoxified RSM in small ruminants, this study aims to evaluate its effects on nutrient intake, digestibility and growth performance in goats.

MATERIALS AND METHODS

Processing of RSM

Sufficient quantity of RSM for the study was supplied by M/s Cardolite Speciality Chemicals Pvt Ltd., Mangalore, India. At laboratory level, raw RSM was processed by soaking, boiling with and without decanting and fermentation for different time periods. Two hundred grams of raw RSM was soaked in water for 24 h in two closed containers. Then, the supernatant was discarded and the samples were dried in an oven at 100±2° C for overnight to obtain soaked RSM. Another two samples, each weighing two hundred grams of raw RSM were placed in separate cooking containers where the water had reached a temperature of 100° C and then boiled separately for 30 minutes and 45 minutes. Boiled samples were allowed to cool and samples were wrapped in aluminum foil and kept in an air tight container and fermented for 7 days. These fermented samples were dried in hot air oven at 100±2° C for overnight. Next two samples, each weighing two hundred grams of raw RSM were placed into separate cooking containers where the water had reached a temperature of 100° C and then one boiled for 30 minutes and another for 45 minutes and samples were dried in an oven at 100±2° C for overnight to obtain boiled without decanting RSM. Another two samples, each weighing two hundred grams of raw RSM were placed in separate cooking containers where the water had reached a temperature of 100° C and then boiled separately for 30 minutes and 45 minutes and supernatant was discarded and samples were dried in an oven at 100±2° C for overnight to obtain boiled with decanting RSM. Finally the products obtained were milled as soaked, boiled cum fermented, boiled and boiled with decanted RSM, respectively. The raw and processed RSM samples were analyzed for proximate composition, fibre fractions, *in vitro* digestibility, residual HCN and mycotoxins.

Chemical analysis and *in vitro* study

Representative samples of raw and differently processed RSM including other feed ingredients, compounded feed mixtures and roughages used in the experiment were collected for analysis. The ash content was determined by measuring the residue obtained after the samples were incinerated at 600° C for a duration of 3 hours in a muffle furnace. The crude protein content (N x 6.25) was assessed using Gerhardt digestion and distillation unit adhering to macro Kjeldahl standards (AOAC, 2005). Following the procedure outlined in AOAC (2005), the ether extract (EE) content of the samples was determined through extraction with petroleum ether. Fibertherm was used to assess the fibre fractions, neutral detergent fiber (NDF) and acid detergent fiber (ADF), as outlined by Van Soest et al. (1991). Various mycotoxins, including aflatoxin (B1, B2, G1 and G2) were analyzed using a two-dimensional thin-layer chromatographic method based on the approach suggested by Tapia (1985). Hydrogen cyanide was analyzed based on the approach suggested by Beek and De Jong (2011) using HPLC with fluorescence detection.

Further, all the samples were analyzed for *in vitro* true dry matter digestibility (IVTDMD), *in vitro* organic matter digestibility (IVOMD) and neutral detergent fibre digestibility (NDFD) according to Goering and Van Soest (1970) modification of Tilley and Terry (1963) in *in vitro* batch fermentor. For the purpose, approximately 400 mg of dried sample was weighed into F57 Ankom Filter bags and incubated for 48 h in sealed Erlenmeyer flasks with a mixture of Mold's buffer and rumen fluid (4:1 ratio) in triplicate. After incubation, leftover residues in bags were treated with neutral detergent solution in an Ankom200 fiber analyzer, and the remaining dry residues were weighed.

Experimental animals

Fifteen goat kids of about 3 to 4 months of age with comparable body weight were selected from the flock maintained at Livestock Research and Information Centre (Sheep), Nagamangala, Mandya Dist., Karnataka. The kids were divided into three groups of five each and treatments were allocated randomly to individual group. The procedures employed in the animal trial were duly approved by Institutional Animal Ethics Committee (IAEC) of Veterinary College, Hebbal, Bengaluru vide No.

VCH/IAEC/2023/02 as per Article No. 13 of the CPCSEA rules laid down by Government of India.

Experimental diets and feeding management

In accordance with ICAR (2013) guidelines, diets were formulated to fulfill the energy and crude protein requirements for maintenance and growth of the experimental kids. The diets were comprised of sorghum hay (COFS-29) and compounded feed mixtures (CFM). Animals of control (T1) group were fed GNC based CFM and T2 and T3 groups of experimental animals were fed CFM incorporated with boiled RSM to substitute 12.5 and 25 % of GNC on nitrogen equivalent basis, respectively. The ingredient composition of CFM T1 was 57% maize and 40% GNC, CFM T2 was 55% maize, 35% GNC and 7% boiled RSM and CFM T3 was 53% maize, 30% GNC and 14% boiled RSM. The mineral mixture and common salt was included at 2 and 1%, respectively in all the CFMs. The daily requirement of CFM was provided in two equal portions and offered, at 09.00 and 16.00 hours. The sorghum (COFS-29) hay served as the sole source of roughage and offered *ad libitum* to all the animals. To assure good intake, the COFS-29 hay was weighed and provided to each individual kids three times a day at 09.30, 14.00 and 17.00 hours. Clean drinking water was provided to individual kids in a plastic bucket thrice a day. The feeding trial was carried out for fourteen weeks with an initial adjustment period of ten days. The body weights of the animals were taken at weekly interval in the morning before offering any feed and water.

Digestion trial

A digestion trial employing all the animals was conducted for six days during the terminal week of the experiment to assess the digestibility of nutrients by following standard procedures. The representative feed, faecal and ort samples were subjected for proximate analysis (AOAC, 2005) and detergent system of feed analysis (Van Soest et al., 1991). The digestibility of nutrient was calculated as the difference in nutrient intake and outgo and expressed as percentage intake.

Statistical analysis

The data obtained during feeding and digestion trial was analysed statistically (ANOVA) using SPSS version 16.0. Individual differences between means were tested using Tukey 't' test and results were interpreted accordingly (Snedecor and Cochran, 1995).

RESULTS AND DISCUSSION

Chemical composition and *in vitro* digestibility

The mycotoxin analysis of raw and boiled RSM revealed that the concentration (as µg/kg) of aflatoxin B1, B2, G1 and G2 in raw and boiled RSM were 133.8, 1.0, 137.4 & 0.5 and 17.0, 1.6, 96.3 & 3.7, respectively. Romero-Sanchez et al. (2024) observed that the thermal cooking (boiling with water at 100^{o}C for 12 min) significantly reduces the aflatoxin content. The maximum permissible level of aflatoxin in ruminant diet is 20 ppb/kg DM. Thus the inclusion level of BRSM in T2 and T3 at 7 and 14 per cent on w/w basis in the CFM ensured the aflatoxins below the specified limit in the diets. The HCN in raw and processed RSM were below limit of quantification (<0.05 mg/kg). Udo et al. (2016) reported that boiling rubber seeds for half an hour, followed by sun drying, effectively eliminated HCN. Similarly, Agbai et al. (2021) observed that fresh rubber seeds initially contained a substantial amount of HCN (12.41 mg/100 g), but boiling reduced it to 4.71 mg/100 g, and fermentation further lowered HCN to 1.97 mg/100 g and these findings were similar to observations of Farr et al. (2019) and Diptanu and Samanta (2020). RRSM contains oxalates, saponins, phytates, trypsin inhibitor in addition to HCN. Boiling not only reduces HCN but also reduces heat labile toxins such as trypsin inhibitor. Thus boiling makes a better option of processing RRSM in comparison to other detoxification methods (Farr et al. 2019). These studies collectively emphasize the effectiveness of boiling in removing HCN and other heat labile anti-nutritional factors like protease inhibitors from RSM, thereby improving its safety for use as protein supplement in ruminant diets.

Table 1. Chemical composition[1] (% DMB) of different feedstuffs and compounded feed mixtures (CFM)

Attribute	Maize	GNC	RRSM	BRSM	CFM			COFS-29 hay
					T1	T2	T3	
Proximate principles								
Organic matter	96.5	95.4	94.3	93.9	95.6	95.6	95.1	90.2
Crude protein	9.43	39.8	30.9	30.2	22.7	22.0	22.0	8.95
Ether extract	2.92	9.86	17.1	15.1	5.17	6.11	6.89	2.34
Total ash	3.46	4.56	5.72	6.16	4.42	4.40	4.90	9.79
Fibre fractions								
Neutral detergent fibre	15.7	19.3	40.3	41.7	19.6	21.1	23.1	65.9
Acid detergent fibre	6.22	10.4	17.9	18.5	8.72	9.98	10.7	38.6
Hemicellulose	9.49	8.92	22.4	23.2	10.9	11.1	12.5	27.3

The proximate composition and fibre fractions of concentrate feed ingredients, compounded feed mixtures (T1, T2 and T3) and sorghum (COFS-29) hay fed to kids during the growth trial are presented in Table 1 and the *in vitro* digestibility values in Table 2. The proximate composition of boiled RSM was comparable with the values reported by Suprayudi et al. (2015) and Aguihe et al. (2017) except for crude fat. The proximate composition with respect to all nutrients was comparable with the values reported by Mmereole et al. (2008) and Ijaiya et al. (2011) except for ether extract which varied. The ether extract was lower in the present study which might be due to lower levels of oil in RSM and it also depends on the oil extraction method followed. The composition with respect to fibre fractions of RSM was comparable with values reported by Nongyao et al. (1991) and Nguyen and Duong (2003). This indicates that detoxified RSM has considerable amounts of absorbable nutrients than many conventional seed meals and exhibits better alternative for protein supplements in livestock diets. The proximate principles of COFS-29 hay were in agreement with the findings of Iyanar et al. (2015) and Senthilkumar et al. (2009). The fibre fraction composition of COFS-29 hay was comparable with the values reported by Wadhwa et al. (2010). The *in vitro* dry matter digestibility of RSM was in agreement with Jayanegara et al. (2018).

Table 2. *In vitro* digestibility (%) of different feed stuffs and compounded feed mixtures (CFM)

Feedstuff	IVTDMD	IVOMD	NDFD
Maize	93.8	94.2	64.3
GNC	81.8	82.8	79.5
RRSM	92.1	92.5	63.5
BRSM	87.9	88.4	59.6
COFS-29 hay	63.7	44.0	39.7
CFM T1	94.5	94.9	75.0
CFM T2	93.3	93.7	71.3
CFM T3	91.5	91.9	69.9

Dry matter and nutrient intake

The mean intake of DM and other nutrients in different treatment groups are presented in Table 3. The total DMI (as gram per day and per cent body weight) for T1, T2 and T3 groups were found non-significant among the treatment groups. The observed DMI in T1, T2 and T3 groups was comparable with the average daily intakes of DM (T1-2.5, T2-2.8 and T3-2.7 %) as reported by Nguyen and Duong (2003) who used three levels of rubber seed cake replacing 0, 50 and 100 % of groundnut cake in the concentrate supplement and found non-significant difference in DMI in growing goats.

The DMI observed in this study was in agreement with the findings of Chanjula et al. (2011) where rubber seed kernel was included at levels ranging from 20 to 30 % to replace palm seed kernel in goats and found no significant difference in DMI at 20 % inclusion level. However, increasing rubber seed kernel levels beyond 20% resulted in a slight reduction in daily DMI. Simillar observations were noted by Tean et al. (2002) where RSM was incorporated at various levels in the experimental diets of pigs, replacing wheat bran at 0, 10, 20 and 30 % and found non-significant DMI among different treatment groups. Ouppamong et al. (2022) also found that the use of yeast fermented rubber seed kernel in the diet of lactating dairy cows did not affect feed intake when included at 0, 10 and 20 % in concentrate portion of diets. In contrast, the observed values were not in agreement with Udo et al. (2021) who included boiled RSM from 0 – 40 % in CFM (0 %, 20 %, 30 % and 40 % inclusion of boiled RSM in compounded feed mixtures) in West African dwarf bucks.

Intakes of OM, NDF and ADF were not significant, whereas the intakes of CP and EE were found to be significant (P<0.05) among the treatment groups (Table 3). In all the three groups the intake of NDF and ADF was adequate, for optimum ruminal fermentation (Van Soest et al., 1991).

Although there was no significant difference in DMI but the significant ($P \leq 0.05$) difference in CP intake was due to less consumption of COFS-29 hay in RSM based diets T2 and T3 groups compared to control. CP intake through CFM was similar in all the treatment groups but intake through sorghum hay differed significantly among the treatment groups, this could be the reason for significant difference in CP intake. The T3 group of animals consumed higher EE as RSM incorporated CFM has more oil compared to control CFM with GNC.

Table 3. Nutrient Intake during feeding trial in different treatments

Attribute	T1	T2	T3	SEM	P-value
DM					
g per day	307.9	278.8	273.4	6.42	0.061
% of body weight	2.94	2.82	2.68	0.01	0.073
g/kg $W^{0.75}$	52.7^{b}	49.8^{ab}	47.8^{a}	0.77	0.035
Organic matter					
g per day	290.9	263.6	257.2	6.05	0.054
% of body weight	2.77	2.67	2.53	0.01	0.058
g/kg $W^{0.75}$	49.8^{b}	47.1^{ab}	45.0^{a}	0.72	0.026
Crude protein					
g per day	58.9^{b}	52.0^{a}	52.6^{ab}	1.21	0.035
% of body weight	0.56^{b}	0.52^{ab}	0.51^{a}	0.00	0.036
g/kg $W^{0.75}$	10.1^{b}	9.26^{a}	9.19^{a}	1.36	0.014
Ether extract					
g per day	14.1^{a}	14.4^{ab}	16.2^{b}	0.33	0.021
% of body weight	0.13^{a}	0.14^{a}	0.15^{b}	0.00	<0.001
g/kg $W^{0.75}$	2.41^{a}	2.56^{a}	2.83^{b}	0.03	<0.001
NDF					
g per day	98.1	90.4	87.9	3.34	0.200
% of body weight	0.93	0.91	0.86	0.01	0.226
g/kg $W^{0.75}$	16.8	16.2	15.4	0.42	0.160
ADF					
g per day	48.7	45.9	44.4	1.41	0.081
% of body weight	0.46	0.46	0.42	0.01	0.121
g/kg $W^{0.75}$	8.36	8.23	7.62	0.19	0.064

[ab]Mean values in a row bearing different superscripts differ significantly (P<0.05 / P<0.01)

Body weight gain and feed conversion ratio

The average daily body weight gain (ADG) and feed conversion ratio (FCR) for three treatment groups during the growth trial is presented in Table 4. In the present study, there was no significant difference in the initial body weight, final body weight and ADG of the kids among the treatment groups. The observations are in corroboration with results of Nguyen and Duong (2003) who found non-significant difference among the treatment groups. In the present study goat kids were fed under intensive system of rearing which might have caused stress as these animals are primarily browsers and need more flexibility in feeding practices. Although, diet (CFM and COFS-29 hay) for the treatment groups (T1, T2 and T3) in the present study was formulated to achieve a target ADG of 75 g in non-descriptive goats (ICAR, 2013), individual variations were observed with respect to intake, digestibility and efficiency of utilization in goat kids which are generally sensitive to any change in feeding regimen.

Table 4. Mean body weights, average daily gain (ADG) and feed conversion ratio (FCR) of experimental kids during the growth trial

Attribute	T1	T2	T3	SEM	P-value
Initial body weight (kg)	9.5	9.28	9.68	0.53	0.960
Final body weight (kg)	12.7	11.7	11.9	0.62	0.837
Body weight gain (kg)	3.16	2.46	2.23	0.26	0.366
Overall ADG (g)	32.2	25.1	22.8	3.36	0.490
Overall FCR	4.00	3.85	4.07	0.73	0.993

There was no significant difference in ADG among the treatment groups which indicates that replacing GNC with boiled RSM on nitrogen equivalent basis did not affect the growth performance of kids. However, negative ADG in both GNC and boiled RSM based diets in a few weeks of the study was primarily due to low DMI and associated stress in stall fed conditions. In the present study, FCR (T1: 4.00, T2: 3.85 and T3: 4.07) was found to be non-significant among the treatment groups which indicates that replacing GNC with boiled RSM did not affect the feed efficiency of growing kids.

Apparent digestibility of DM and its nutrients

There was no significant difference in the apparent digestibility of different nutrients viz. DM, OM, CP, EE, NDF and ADF among the experimental groups (Table 5). In the present study apparent digestibility of DM, OM, CP, EE, NDF, ADF and digestible organic matter in dry matter (DOMDM %) were similar in all the groups indicating no adverse effect on digestibility of nutrients (Table 5). The nutrient digestibility (%) values for T1, T2 and T3 are in agreement with the reported values by Vishwanathan (1977) in growing calves for digestibility for DM, OM, CP and EE. The results are comparable with the findings of Chanjula et al. (2011) who found non-significant difference among the treatment groups in the digestibility of DM, OM and CP. However, the apparent digestibility of NDF, ADF and EE differed significantly ($p \leq 0.05$) among treatment groups and such a trend was not observed in the present study. The apparent digestibility co-efficient of DM and EE in goats were in corroboration with the findings of Udo et al. (2021) who found non-significant difference among the treatment groups.

Table 5. Apparent digestibility (%) of different nutrients during the digestion trial

Attribute	T1	T2	T3	SEM	P-value
DM	73.4	76.9	76.8	0.02	0.180
OM	75.4	78.7	78.3	0.01	0.201
CP	70.0	72.4	71.6	0.01	0.637
EE	70.4	74.8	75.0	0.01	0.130
NDF	46.0	52.3	52.0	0.01	0.120
ADF	31.2	37.6	36.1	0.03	0.219
DOMDM[1]	71.4	74.6	73.7	0.01	0.219

[1] digestible organic matter in dry matter

CONCLUSION

Considering the overall performance of the experimental kids, feeding of boiled rubber seed meal based diets resulted in similar nutrient intake and digestibility, body weight gain and feed conversion ratio as compared to groundnut cake based diet. It is inferred that, boiled rubber seed meal can be utilized as a component of compounded feed mixtures up to 25 % of the conventional protein source on nitrogen equivalent basis for feeding ruminants to ensure efficient utilization of available non-conventional feed resources and reduce the cost of livestock production.

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