B-5256
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RESEARCH ARTICLE   Indian Journal of Animal Research

In vitro Evaluation of Different Levels of Cashew (*Anacardium occidentale*) Nut Meal Supplementation on Rumen Fermentation Kinetics and Digestibility

K.M. Rashmi[1], T.M. Prabhu[1], Vivek M. Patil[2], H.S. Madhusudhan[1], N.M. Soren[3], K.S. Giridhar[4], Hemantkumar Pandey[5]

10.18805/IJAR.B-5256

ABSTRACT

Background: A shortage of feed resources poses challenges for sustainable livestock production. Researchers are increasingly investigating alternative feeds to replace conventional ones. Cashew nut meal (CNM), a by-product of the cashew nut kernel industry, is a promising alternative feed for ruminants owing to its good nutritive value. Due to limited research on optimal inclusion levels in ruminant diet, this study aimed to evaluate the effect of incorporating varying levels of CNM as a supplement on *in vitro* rumen fermentation kinetics and digestibility.

Methods: Seven distinct compounded feed mixtures (CFM) were formulated, with CNM progressively replacing 0 (C0), 10 (C1), 20 (C2), 30 (C3), 40 (C4), 50 (C5) and 60 (C6) per cent of the soybean meal (SBM) protein present in the control CFM. Additionally, seven experimental complete diets (T0 to T6) were prepared by blending these CFM with Super Napier (*Pennisetum purpureum* × *Pennisetum glaucum*) hay in a 40:60 ratio. These CNM based complete diets were subjected to rumen *in vitro* gas production (IVGP) study with cumulative gas production (GP) measured at 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72 and 96 h post-incubation. Subsequently, *in vitro* true dry matter digestibility (IVTDMD) and neutral detergent fiber digestibility (NDFD) of the diets were determined using a modified *in vitro* two-stage technique. Later, total volatile fatty acids (TVFA) were estimated using gas chromatography.

Result: Analysis of chemical composition revealed that CNM contains good protein of 256.5 g/kg. The potential gas production (D) and the rate and extent of gas production (c) for diets containing CNM ranged from 54.63 to 60.24 mL and 0.036 to 0.043 h^{-1}, respectively. IVTDMD and NDFD analysis of the seven complete diets fell within the range of 79.12 to 80.80% and 64.72 to 66.47%, respectively. The estimated TVFA for seven complete diets ranged from 17.87 to 23.65 mM. Further, the metabolisable energy (ME) of diets ranged from 7.89 to 8.08 MJ/kg DM. Importantly, no significant differences were observed in rumen fermentation kinetics parameters, IVTDMD, NDFD and TVFA among treatments (T0 to T6). CNM could be used as an alternative to SBM in compounded feed mixtures of ruminants up to 30% (w/w) without any adverse effect on rumen fermentation pattern and digestibility.

Key words: Cashew nut meal, *In vitro* digestibility, Rumen fermentation kinetics, Super napier.

INTRODUCTION

The availability of sufficient, high-quality feed resources stands as a major constraint to achieve sustainable livestock production (Singh *et al*., 2015). Meanwhile, the task of ensuring food and nutritional security for an ever-expanding human population necessitates the establishment of sustainable crop and livestock production systems. In the context of a growing livestock population, there persists a noteworthy scarcity, with deficits of 35.6% for green feed, 10.95% for dry feed and a substantial 44% deficit in concentrate feed in India (IGFRI, 2015). This situation is further exacerbated by factors such as rapid urbanization, a reduction in available cultivable land and intense competition among the feed, fuel and food sectors. Additionally, the escalating prices of conventional feedstuffs have propelled the search for alternative options. The potential solution lies in the exploration and utilization of unconventional feed resources in addition to the judicious use of existing feed sources. In this perspective, CNM, a by-product of cashew processing, holds promise as an alternative protein source for ruminants. With considerable

[1]Department of Animal Nutrition, Veterinary College, KVAFSU, Bengaluru-560 024, Karnataka, India.
[2]Department of Livestock Production and Management, Veterinary College, KVAFSU, Bengaluru-560 024, Karnataka, India.
[3]ICAR-National Institute of Animal Nutrition and Physiology, Adugodi, Bengaluru-560 030, Karnataka, India.
[4]Department of Animal Nutrition, Veterinary College, KVAFSU, Bidar-585 403, Karnataka, India.
[5]Alltech, KVAFSU-Alltech Research Alliance, Bengaluru-560 024, Karnataka, India.

Corresponding Author: T.M. Prabhu, Department of Animal Nutrition, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bengaluru-560 024, Karnataka, India. Email: prabhutmann@gmail.com

How to cite this article: Rashmi, K.M., Prabhu, T.M., Patil, V.M., Madhusudhan, H.S., Soren, N.M., Giridhar, K.S. and Pandey, H. (2024). *In vitro* Evaluation of Different Levels of Cashew (*Anacardium occidentale*) Nut Meal Supplementation on Rumen Fermentation Kinetics and Digestibility. Indian Journal of Animal Research. doi: 10.18805/IJAR.B-5256.

Submitted: 23-10-2023 **Accepted:** 30-01-2024 **Online:** 26-02-2024

availability, cost-effectiveness and minimal competition for human consumption, CNM has recently gained attention.

The cashew (*Anacardium occidentale*) tree is an evergreen tree belonging to the family *Anacardiaceae*. The tree is cultivated for its fruit (cashew nut) and pseudofruit (cashew apple). During agro-industrial processing of cashew kernels, approximately 30 to 40% of cashew nuts are deemed unfit for human consumption and subsequently discarded due to their failure to meet minimum quality standards. These discarded cashew nuts have good nutritive value, containing 18 to 27% protein and 36 to 51% oil (Heuzé *et al*., 2017). The oil is rich in unsaturated fatty acids like oleic acid (60%) and linoleic acid (22%) (Rico *et al*., 2015; Pereira *et al*., 2016). It also contains essential minerals such as calcium, sodium, phosphorus, potassium, magnesium, iron and zinc (Silué *et al*., 2017). The CNM may serve as an alternative to conventional protein sources for cost- effective feeding. Therefore, this study aims to assess the effect of supplementing various inclusion levels of CNM on *in vitro* rumen fermentation kinetics and digestibility.

MATERIALS AND METHODS

The present study was carried out at the Department of Animal Nutrition, Veterinary College, Bengaluru during 2022-23 to evaluate the effect of cashew nut meal supplementation on *in vitro* rumen fermentation kinetics and digestibility.

Sample collection and preparation

Samples of discarded cashew nut (DCN) and cashew nut meal (CNM) were collected from Ramanjaneya mill, Virudhunagar, Tamil Nadu. These samples were sun-dried for two days. Additionally, the remaining feed samples, such as maize, soybean meal, wheat bran and Super Napier (SN) hay, were ground to pass through a 1 mm sieve and preserved for future experiments. SN hay served as the primary roughage source in the study.

Chemical analysis

Feed samples were analyzed for their proximate composition as per AOAC (2005) and the fibre fractions were determined according to Van Soest *et al*. (1991).

Experimental diets

Seven iso-nitrogenous CFM were prepared by incorporating graded level of CNM replacing 0, 10, 20, 30, 40, 50 and 60% CP from SBM of the control CFM (C0 to C6) (Table 2). Subsequently seven iso-nitrogenous experimental complete diets (T0 to T6) were prepared by mixing these CFM with SN hay in ratio 40:60 for *in vitro* studies.

Assessment of biological parameters

This study involved the assessment of three biological parameters: IVGP, IVTDMD and NDFD. IVGP was analysed according to the method delineated by Menke and Steingass (1988), while the other two parameters were determined using a modified *in vitro* two-stage technique (Goering and Van Soest, 1970).

In vitro gas test

Air-equilibrated feed substrates (200±10 mg) were incubated at 39°C for 96 hours with 30 mL mixed rumen inoculum (rumen buffer and rumen liquor in a 2:1 ratio) (Menke and Steingass, 1988), using 100 mL calibrated syringes in triplicate. Rumen liquor was collected from donor cow fed on roughage (Finger millet straw) and CFM based diet to meet nutrient (DM, CP and TDN) requirements for maintenance (ICAR, 2013). Cumulative GP was recorded at 0, 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72 and 96 h of incubation and data were fitted to the exponential equation:

$$Y=a+b\ (1-e^{-ct})$$

Where,

Y = Represented GP (mL) at time t.

a = Denoted the initial GP (mL).

b = Indicated the GP (mL) during incubation.

a+b or D = Represented the potential GP (mL).

c = Fractional GP rate (mL/h).

The ME, *in vitro* organic matter digestibility (IVOMD) and short chain fatty acids (SCFA) of samples were determined according to Menke and Steingass (1988).

Partitioning factor (PF) is the ratio of true organic matter degraded (mg) to the volume of gas produced (mL) during 24 h incubation (Blummel *et al*., 1997a). The microbial biomass production (MBP) was estimated according to the equation elucidated by Blummel (2000).

$$MBP = TOMD - (GV \times SF)$$

Where,

TOMD = True organic matter degraded during 24 h.

SF= Stoichiometric factor = 2.2.

GV= Gas volume during 24 h.

In vitro true dry matter digestibility (IVTDMD) and Neutral detergent fibre digestibility (NDFD)

IVTDMD analysis was conducted with *in vitro* batch fermentor. Approximately 400 mg (2 mm) dry samples were weighed in 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). After incubation, leftover residues in bags were treated with Neutral Detergent Solution in an Ankom200 fiber analyzer and the remaining dry residues were weighed. The flask contents were checked for pH, transferred to centrifuge tubes and centrifuged at 5000 rpm for 20 minutes at 4°C. The supernatant (800 μl) was mixed with 25% metaphosphoric acid (200 μl) and stored at -20°C for subsequent VFA analysis. The concentration of VFA were determined using a gas chromatograph (Agilent; Model 7890 A GC System) equipped with a flame ionization detector and a Agilent J and W DB-WAX GC column (Filipek and Dvorak, 2009). All VFA were corrected for blank. The TVFA was calculated by summing up the concentration of all the individual VFA. IVTDMD and NDFD were determined as per Goering and Van Soest (1970).

Statistical analysis

The data were subjected to one way analysis of variance (ANOVA) as per Snedecor and Cochran (1994). The data were analysed using SPSS (2008) version 15.0 and the significance of differences between means were assessed using Tukeys test.

RESULTS AND DISCUSSION

The chemical composition of protein supplements and SN hay is detailed in Table 1. DCN (CP - 241.6 g/kg) and CNM (CP - 256.5 g/kg) contain a substantial amount of protein, although lower when compared to SBM (452 g/kg). CNM (111g/kg) exhibited a lower ether extract content compared to DCN (377.7 g/kg) but higher than SBM (11.7 g/kg). Additionally, CNM had higher proportions of total carbohydrates (563.5 g/kg) and fibrous fractions, including NDF (533.2 g/kg), ADF (353.7 g/kg) and ADL (61.0 g/kg), in comparison to SBM and DCN. The CP and EE values of DCN were consistent with the findings of previous studies (Akande *et al*., 2015; Rico *et al*., 2015; Abubakar *et al*., 2018). The total carbohydrate (TCHO) content of DCN was in agreement with the findings of Abubakar *et al*. (2018). However, CNM displayed lower CP content compared to the results of Akande *et al*. (2015) and Coffi *et al*. (2023). This disparity could potentially be attributed to variations in the oil extraction methods employed in these studies. The current study utilized the meal from a traditional cold press unit for oil extraction from discarded cashew nuts, acknowledged as less efficient compared to other methods of oil extraction. While the chemical composition of CNM is in corroboration with the values reported by Sravani *et al*. (2021). Although DCN contain a substantial amount of protein, its higher EE (377.7 g/kg) restricts its utilization in ruminant diets due to the potential for causing rumen disturbances resulting from elevated fat content (Donald, 1994). Consequently, the substantial crude protein content and lower EE in CNM position it as an appealing alternative to conventional protein supplements for ruminants.

The chemical analysis of the seven CFM, in which SBM CP was replaced at levels of 0, 10, 20, 30, 40, 50 and 60% by CNM, revealed that the CP content across these mixtures ranged from 208.1 to 208.3 g/kg (Table 2). This consistent range indicates an isonitrogenous condition, indicating minimal variability in the chemical composition among the different mixtures. Further, the seven isonitrogenous experimental complete diets (CP-144.1 to 144.2 g/kg) were prepared by mixing CFM with SN hay in a ratio of 40:60. The chemical constituents of the diets such as EE, NDF, ash and TCHO ranged from 21.5 to 32.4, 530.98 to 559.3, 100.7 to 104.8 and 722.6 to 729.4 g/kg, respectively.

The data on *in vitro* gas profile of SBM and CNM is summarized in Table 3. It was observed that the GP potential of CNM (54.69 mL/0.2 g DM) was similar to that of SBM (54.77 mL/0.2 g DM). The GP is positively correlated with SCFA production (Blummel *et al*., 1997a), as evidenced by the SCFA produced with CNM (1.21 mmol/0.2 g DM) being comparable to that of SBM (1.21 mmol/0.2 g DM). However, IVOMD of CNM (79.50%) was higher but lower as compared to SBM (89.61%) which might be due to higher content of structural carbohydrates in CNM compared to SBM (McDonald, 2002; Purwin *et al*., 2016). It is important to note that the IVOMD values for both CNM and SBM in this study were high, as supported by Sutardi (1980), who regarded values exceeding 70% as high digestibility. The partitioning of fermented organic matter between microbial biomass and GP is not uniform (Blümmel *et al*., 1997b). The PF values obtained for SBM (3.27) and CNM (2.91) in this study fell within the theoretical range 2.75 to 4.41 (Blümmel *et al*., 1997b) and lower PF values of CNM compared to SBM is attributed to their lower digestibility compared to SBM. Furthermore, estimated ME values for CNM (13.68 MJ/kg DM) were higher than those for SBM (12.99 MJ/Kg DM). These differences in energy values in spite of similar GP, appeared to be related to variations in the chemical composition (CP, EE, TA) of the feeds, as the formula for estimating ME takes these individual chemical constituents into account.

Table 1: Chemical composition (g/kg DM) of feedstuff.

Composition	SBM	CNM	DCN	SN hay
DM	890	906.3	953.7	880
OM	911.3	931.0	956.5	872.3
CP	452	256.5	241.6	99.6
EE	11.7	111	377.7	20.2
Ash	88.7	69	43.5	127.7
AIA	20.1	3.5	2.9	52.4
NDF	175.5	533.2	274.3	727.5
ADF	130.1	353.7	114.4	474.4
ADL	34.9	61	24	69.7
Cellulose	93.9	291.9	89.9	403.3
Hemicellulose	45.4	179.5	159.9	253.1
TCHO*	447.6	563.5	337.2	752.5

SBM: Soybean meal; CNM: Cashew nut meal; DCN: Discarded cashew nut; SN hay: Super Napier hay; T-CHO*: Total carbohydrate.

Table 2: Ingredient and chemical composition of cashew nut meal based compounded feed mixtures.

Attribute	Compounded feed mixtures						
	C0	C1	C2	C3	C4	C5	C6
				Ingredient composition (g/kg, as mixed)			
Maize grain	419.0	419.0	419.0	419.0	419.0	417.3	414.0
Soybean meal	285.0	266.0	228.0	196.9	171.0	142.0	114.0
Wheat bran	266.0	234.8	220.6	200.2	175.2	154.7	134.9
Cashew nut meal	0	50	100	150	200	250	300
Mineralmixture	20	20	20	20	20	20	20
Common salt	10	10	10	10	10	10	10
Urea	0.0	0.2	2.4	3.9	4.8	6.0	7.1
				Chemical composition (g/kg)			
DM	871.9	872.3	873.1	873.8	874.3	874.9	875.5
OM	929.7	932.1	933.4	933.5	935.3	938.6	940.0
CP	208.3	208.1	208.1	208.2	208.1	208.2	208.0
EE	23.5	27.9	32.5	37.1	41.6	46.1	50.7
TA	70.3	67.9	66.6	66.5	64.7	61.4	60.0
AIA	26.9	24.6	23.0	21.2	19.2	17.4	15.6
NDF	235.7	245.1	258.8	270.9	281.9	294.1	306.5
ADF	109.9	119.7	130.0	140.2	150.2	160.4	170.9
ADL	21.7	22.9	24.1	25.4	26.6	27.9	29.3
Hemicellulose	125.8	125.4	128.8	130.7	131.7	133.6	135.6
Cellulose	88.2	96.7	105.9	114.8	123.5	132.5	141.6
TCHO	698.2	696.2	692.8	688.2	685.4	684.2	681.2

Table 3: *In vitro* gas production and metabolisability of protein supplements.

Parameters	SBM	CNM
GP 24 h (mL/0.2 g DM)	54.77	54.69
IVOMD (%)	89.61	79.50
MBP (mg)	293.6	193.4
PF	3.27	2.91
SCFA (mmol/0.2g DM)	1.21	1.21
ME (MJ/kg DM)	12.99	13.68
IVTDMD (%)	94.82	82.56
NDFD (%)	78.01	71.12
TVFA (mM)	26.89	20.55
Acetate (%)	46.05	58.72
Propionate (%)	24.30	26.45
Butyrate (%)	13.70	11.26

GP_{24h}, gas production for 24 h; MBP, Microbial biomass production; PF, Partitioning factor; SCFA, Short chain fatty acids; IVOMD- Calculated *in vitro* organic matter digestibility; ME- Metabolisable energy; IVTDMD- *In vitro* true dry matter digestibility measured at 48 h incubation; NDFD- Neutral detergent fibre digestibility measured at 48 h incubation; TVFA-Total volatile fatty acids measured at 48 h incubation.

The IVTDMD and NDFD of CNM (82.56%, 71.12%) were lower compared to SBM (94.82%, 78.01%), likely due to the higher structural carbohydrate content of CNM (McDonald *et al*., 2002). However, CNM and SBM both had high IVTDMD and NDFD (above 70%) (Sutardi, 1980). Further, the lower IVTDMD and NDFD of CNM led to lower TVFA in CNM (20.55 mM) compared to SBM (26.89 mM). The proportions of acetic acid (58.72%), propionic acid (26.45%) and butyric acid (11.26%) were higher for CNM which might be due to its higher NDF (533.2 g/kg) content. Furthermore, the ratio among acetate, propionate and butyrate observed with CNM was 60:30:10, which is a typical ratio commonly found in the rumen (Bergman, 1990).

In vitro rumen fermentation kinetics parameters of all seven complete diets are presented in Table 4 and Fig 1. The potential GP (D) ranged from 54.63 to 60.24 mL and half-life ($t_{1/2}$) for these diets varied between 16.21 and 19.56 h. Further, the rate of GP (c) for diets ranged from 0.036 to 0.043 h^{-1}. The k for T5 and T6 was lower (P>0.05) compared to other diets which might be due to the negative correlation of k with NDF content of diets. In addition, higher inclusion levels of CNM in diets T5 and T6 contributes to higher fibrous carbohydrates compared to other diets, which might be responsible for their higher (P>0.05) $t_{1/2}$ (Kim and Sung, 2022). Interestingly, different levels of CNM inclusion in the diets did not exert any significant (P>0.05) influence on rumen fermentation kinetics parameters. This lack of effect could be attributed to the similar TCHO content among the diets and supported by the fact that GP primarily results from the fermentation of carbohydrates in the diets.

Rumen fermentation by anaerobic microbes generates SCFA, gases and microbial biomass. Measuring GP during incubation predicts feed digestion (Mohamed and Chaudhry, 2008). The measured GP of seven diets (T0 to T6) at 24 h, ranged from 37.34 to 38.15 mL/0.2 g DM (Table 4). The SCFA and ME of diets ranged from 0.838 to 0.843 mmol/ 0.2g DM and 7.89 to 8.08 MJ/kg DM, respectively. Nonetheless, there were no significant (P>0.05) differences observed for GP 24 h, SCFA and ME among the diets. Furthermore, the IVOMD, MBP and PF for the seven diets ranged from 61.14 to 62.09%, 199.84 to 201.26 mg and 3.25 to 3.28, respectively (Table 4). Notably, the T0 diet, having higher IVOMD (P>0.05), exhibited higher (P>0.05) MBP compared to the other diets. This phenomenon may be attributed to the positive relationship between IVOMD and MBP (Blummel *et al*., 1997b). However, the differences

Table 4: *In vitro* rumen fermentation kinetics of cashew nut meal based complete diets.

Parameter	Complete diets							SEM	P value
	T0	T1	T2	T3	T4	T5	T6		
D	59.11	55.19	54.63	55.81	55.32	58.28	60.24	0.665	0.114
b	59.60	57.08	55.97	57.81	57.79	58.23	60.13	0.410	0.066
k	0.039	0.043	0.040	0.039	0.039	0.036	0.036	0.001	0.141
$t_{1/2}$	17.69	16.21	17.22	17.61	17.71	19.46	19.56	0.377	0.176
GP 24 h (mL/0.2 g DM)	38.15	37.95	37.83	38.07	38.17	37.34	38.10	0.284	0.989
IVOMD (%)	62.09	61.86	61.71	61.93	61.97	61.14	61.97	0.252	0.985
MBP (mg)	201.26	201.06	200.96	200.44	199.84	200.65	199.84	0.607	0.842
PF	3.26	3.26	3.27	3.26	3.25	3.28	3.25	0.011	0.981
SCFA (mmol/0.2 g DM)	0.84	0.84	0.84	0.84	0.84	0.82	0.84	0.006	0.989
ME (MJ/kg DM)	7.89	7.90	7.93	8.01	8.07	7.99	8.08	0.046	0.096

D: Potential GP; b: GP from insoluble fractions; c: Rate constant; $t_{1/2}$: Half life; Complete diets: T_0 to T_6 were prepared with CNM based CFM (0, 5, 10, 15, 20, 25 and 30 %) and super napier hay in 40:60 proportion.

Table 5: *In vitro* true digestibility and volatile fatty acid profile of cashew nut meal based complete diets.

Parameter	Complete diets							SEM	P value
	T0	T1	T2	T3	T4	T5	T6		
IVTDMD (%)	80.80	80.09	79.71	79.12	80.03	79.66	80.12	0.001	0.174
NDFD (%)	66.47	66.19	64.72	63.99	66.12	65.42	66.36	0.002	0.349
pH	6.65	6.71	6.70	6.67	6.72	6.73	6.69	0.008	0.402
TVFA (mM)	23.65	19.59	20.51	22.36	18.10	17.87	22.19	2.430	0.582
Acetate (%)	50.77	48.45	48.98	49.34	45.56	45.29	48.10	0.780	0.569
Propionate (%)	28.03	29.23	31.94	32.57	35.02	37.48	35.38	0.792	0.311
Butyrate (%)	15.41^{a}	16.32^{ab}	13.64^{ab}	13.23^{ab}	13.63^{ab}	12.07^{b}	11.80^{b}	0.360	0.033
Isobuytrate(%)	1.18	1.10	0.94	0.98	1.00	0.88	0.98	0.047	0.797
Valerate (%)	2.33	2.69	2.51	2.14	2.80	2.63	2.18	0.163	0.824
Isovalerate (%)	2.29	2.21	1.99	1.74	1.99	1.65	1.55	0.085	0.438

^{a,b} Values with different superscripts within a row indicate significant difference at p<0.05.

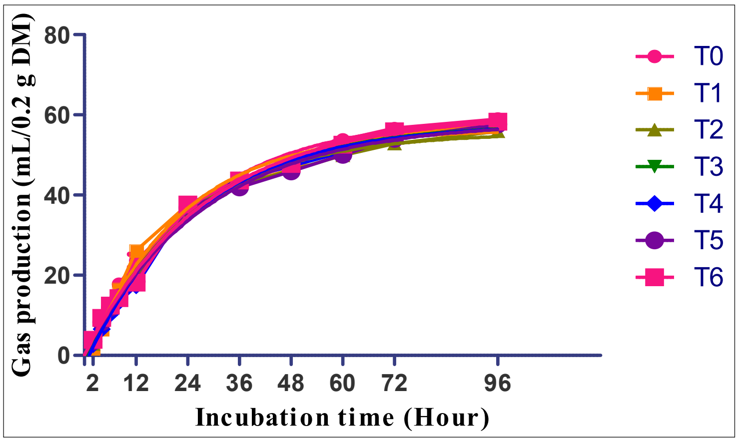

Fig 1: *In vitro* gas production of cashew nut meal based complete diets.

observed in these above parameters remained statistically similar among the diets.

Further, IVTDMD and NDFD of seven complete diets ranged from 79.12 to 80.80and 64.72 to 66.47%, respectively (Table 5). The non-significant differences observed in IVTDMD and NDFD of diets might be due to similar structural carbohydrate contents of diets. The TVFA for seven diets varied from 17.87 to 23.65 mM (Table 5), but remained statistically similar among the diets. With addition of CNM, proportion of acetate, butyrate, isobutyrate and isovalerate decreased (P>0.05) while propionate increased (P>0.05) (Table 5). At higher inclusion levels of CNM in diet T5 and T6, there was decrease (P<0.05) in butyrate.

The rumen pH is the indicator of effect of TVFA (Zhang *et al*., 2022), in this regard T0 diet with higher TVFA (23.65 mM) has lower pH (6.65) while lower concentration of TVFA in T5 (17.87 mM) resulted in slightly higher pH (6.73). Interestingly, different inclusion levels of CNM did not result in alterations (P>0.05) in rumen pH. The pH values (6.65 to 6.71) fell within the normal physiological range of 6.2 to 7.0, considered essential for optimum functioning of cellulolytic bacteria for efficient digestion of fibrous materials (Orskov and Ryle, 1990).

CONCLUSION

Based on the findings related to the chemical composition, gas production and digestibility, it appears that cashew nut meal can be used as a replacement for soybean meal, up to 30% in concentrate mixture. However, animals' trials are needed to confirm and validate the results obtained from the *in vitro* experiments.

ACKNOWLEDGEMENT

First author sincerely acknowledges the Karnataka Science and Technology Promotion Society (KSTePS), Department of Science and Technology, Govt. of Karnataka for providing financial support through DST- Ph.D. fellowship throughout this study.

Conflict of Interest

All authors declare that they have no conflicts of interest.

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