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Potential of *Cassia tora* Seed Meal as an Alternative Protein Source in Total Mixed Rations of Ruminants: An *In vitro* Study

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ABSTRACT

The objective of the present study was to evaluate the *in vitro* nutritional worth of total mixed rations (TMRs) containing graded levels of *Cassia tora* seed meal. Six TMRs (designated as TMR 1, TMR 2, TMR 3, TMR 4, TMR 5 and TMR 6) were formulated with graded inclusion levels of *C. tora* seed meal in the concentrate mixture (0, 4, 6, 8, 10 and 12%, w/w) The concentrates were mixed with maize silagein R:C ratio of 50:50 to formulate TMRs. *In vitro* evaluation revealed that nutrient digestibility (OM, NDF, DM) of TMRs did not differ significantly (P>0.05) across the treatments. The NGP production, SCFA concentration and ME availability remained comparable among TMRs containing *C. tora* seed meal up to 8% inclusion level, while significantly lower (P<0.05) values were recorded in TMR 5 and TMR 6 containing 10% and 12% inclusion level of *C. tora* seed meal, respectively, i.e. a significant decline (P<0.05) in these parameters was observed beyond 8% inclusion level. NH_3-N concentration decreased progressively with increasing levels of *C. tora* seed meal, with significantly lower (P<0.05) values observed in TMR 5 and TMR 6. *In vitro* results indicated that *C. tora* seed meal could be incorporated safely up to 8% in the concentrate mixture of TMRs without adverse effects on nutrient digestibility, while higher inclusion levels (beyond 8%) reduced (P<0.05) SCFA production and ME availability.

HIGHLIGHTS

- Effects of adding graded levels of *C. tora* seed mealin the TMRs were studied *in vitro*.
- Higher inclusion levels (10% and 12% on w/w basis in concentrate) reduced (P<0.05) net gas production, short chain fatty acids and ME availability.

Keywords: *Cassia tora* seed meal, *In vitro* evaluation, Total mixed ration

India supports a large livestock population (536.76 million) relative to its available land resources, while only a small fraction of cultivable land is committed to fodder production. Consequently, deficits of green fodder (35.6%), dry fodder (10.95%), and concentrate feeds (44%) have been reported, with projections indicating that these shortages are likely to persist in the future due to increasing competition between food crop cultivation and livestock feeding (Singh *et al*., 2022; IGFRI Vision, 2050). In addition, the escalating cost and limited availability of conventional protein sources such as soybean meal and oil cakes pose economic challenges for smallholder farmers. Under these conditions, there is growing interest in identifying alternative, non-conventional feed resources that are locally available, cost-effective, and do not compete directly with human feed (Singh, 2018). One such potential feed ingredient is *Cassia tora* Linn., an annual leguminous plant widely distributed in tropical

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regions. The seeds of *Cassia tora* are reported to contain appreciable levels of crude protein, essential amino acids, indicating their suitability as a protein source in ruminant diets (Pawar and D'Mello, 2011; Kamble and Deshmukh, 2020). However, information on the utilization of *Cassia tora* seed meal when incorporated into total mixed rations (TMRs), and its effects on rumen fermentation characteristics remains limited.

In view of the limited information available, the present experiment was conducted with the objective of assessing the suitability of *Cassia tora* seed meal as an alternative protein source for ruminants.

MATERIALS AND METHODS

The present study was conducted in the Department of Animal Nutrition, GADVASU, Ludhiana. The research was approved by the Institutional Animal Ethics Committee. The *C. tora* seed meal was procured from local market.

Experimental diets and treatments

TMR had a roughage to concentrate ratio of 50:50 where roughage used was maize silage. Conventional concentrate mixture used in TMR contained maize 40, soybean meal 22, wheat bran 15, rice polish 7, deoiled rice bran 13, mineral mixture 2 and salt 1 part each. *C. tora* seed meal was incorporated in the experimental concentrate mixtures at graded levels of 0, 4, 6, 8, 10 and 12 % (w/w) replacing soybean meal on N basis.

Chemical analysis

Maize silage based TMRs were analyzed for proximate (AOAC, 2007) and cell wall constituents (Van Soest *et al*., 1991).

In vitro evaluation (Menke *et al.,* 1979)

Rumen liquor was obtained from rumen-fistulated male buffaloes maintained on a basal diet consisting of 2 kg of a conventional concentrate mixture (maize 37, mustard cake 12, soybean meal 11, deoiled rice bran 27, rice polish 10, mineral mixture 2 and common salt 1 part), along with 8 kg green fodder, 1 kg wheat straw and ad libitum access to a urea molasses mineral block. Rumen contents were collected into a pre-warmed double-walled (Thermos) flask, flushed with CO_2, and maintained at 39°C. The collected rumen contents were homogenized using a blender for 2 to 3 minutes while maintaining the temperature at 39°C, and subsequently filtered through four layers of muslin cloth. CO_2 was continuously passed through a submerged tube during the addition of the reducing solution until the initially bluish solution turned pink and finally became colourless. Upon attaining a colourless state, strained rumen liquor (SRL) was mixed with buffer medium in a 1:2 ratio. Carbon dioxide flushing was continued until the final syringe was filled. The capillary tube of each syringe was securely connected to the bottle top dispenser, and each syringe was filled with 30 ml of the SRL: buffer mixture from a flask kept in a water bath at 39°C. The syringe contents were gently mixed, and air bubbles were removed by carefully moving the piston upward and expelling them through the capillary. The clip was immediately closed, the exact volume recorded, and the syringes were incubated in a water bath maintained at 39°C. During the initial hours of incubation, all syringes were gently swirled at hourly intervals. When gas production exceeded 70 ml after 8 h of incubation, the volume was recorded and the gas was released. Total gas production was measured after 24 h of incubation. Each incubation run included blank syringes in triplicate. After 24 h, syringe contents were transferred to spoutless beakers and refluxed with neutral detergent solution to determine OM and NDF digestibility (Van Soest and Robertson, 1988). Ammonia-nitrogen (NH_3-N) concentration was estimated after 24 h of incubation. Metabolisable energy (ME) was calculated based on the volume of gas produced. The partitioning factor (PF) was calculated as the ratio of truly degraded substrate (mg) to the volume of gas (ml) produced during *in vitro* fermentation (France *et al*., 1993).

Statistical Analysis

Data were analysed by simple ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21. The differences in means were tested by Tukey's b.

RESULTS AND DISCUSSION

Chemical composition of TMRs containing graded levels of *C. tora* seed meal

The chemical composition of the experimental total mixed rations (TMRs) is presented in Table 1. The organic matter (OM) content of the TMRs varied from 92.72 % to 93.37%, indicating uniformity among the rations. The crude protein (CP) content of TMR 1, TMR 2, TMR 3, TMR 4, TMR 5 and TMR 6 was 12.11 %, 12.49 %, 12.16 %, 12.32 %, 12.15 % and 12.01 %, respectively, indicating that all the TMRs were isonitrogenous. The ether extract (EE) content of the TMRs ranged from 3.00 % to 3.30 %, with almost similar values among diets. The total ash content varied between 6.64 % and 7.28 %. The neutral detergent fibre (NDF) concentration in the TMRs ranged from 53.50 % to 56.76 %, whereas the acid detergent fibre (ADF) content varied from 27.45 % in TMR 2 to 30.30 % in TMR 6.

The hemicellulose content of the TMRs ranged between 25.25 % to 28.05 %. The acid detergent lignin (ADL) content showed minimal variation and ranged from 4.70 % to 5.20 % across the rations. The total carbohydrate (TCHO) content of TMR 1, TMR 2, TMR 3, TMR 4, TMR 5 and TMR 6 was 77.61 %, 77.21 %, 77.68 %, 77.58%, 77.91 % and 78.07 %, respectively, indicating similar carbohydrate levels among all the experimental diets.

In vitro evaluation of TMRs containing graded levels of *C. tora* seed meal

The fermentation parameters of TMRs are presented in Table 2. The net gas production (NGP; ml/g DM/24 h) in TMR 1, TMR 2, TMR 3 and TMR 4 was similar with values of 193.33, 194.00, 196.00 and 200.67, respectively, whereas NGP was significantly lower ($P<0.05$) in TMR 5 (184.00) and TMR 6 (182.67). The results of the present study are in accordance with those of Akinbode *et al*. (2023) who demonstrated that increasing inclusion of *Cassia fistula* leaf powder from (0 to 60 mg/g DM levels) linearly reduced total gas production (24 h: 41 to 28 ml, $P=0.002$; linear $P<0.001$; 48 h: 59.6 to 51.3 ml, $P<0.001$) and methane output (20 to 9 ml/200 mg DM, $P=0.005$; linear $P<0.001$), achieving a 46% methane reduction. Our results are contrary to those of Vansola *et al*. (2025) who reported that 2% inclusion of *Senna gardneri* (leguminous plant related to genus *Cassia*) significantly reduced ($P<0.05$) net gas (9.00±0.71 ml) and methane production (1.17±0.24 ml CH_4/100 mgDM) compared with the control, whereas higher inclusion levels (6, 7 and 8 %) increased total gas output. In another study, Wahyuni *et al*. (2024) reported that leaf extracts of *Cassia* species significantly increased ($P<0.05$) total gas production while reducing methane output ($P<0.05$), with *C. surattensis* lowering methane from 18.3 to 16.9 ml/200 g substrate and methane per unit of digestible organic matter from 38.4 to 33.0 ml/g.

Table 1: Chemical composition of TMRs containing graded levels of *C. tora* seed meal, % DM basis

Parameter	TMR 1	TMR 2	TMR 3	TMR 4	TMR 5	TMR 6
OM	92.72	92.81	92.99	93.00	93.37	93.23
CP	12.11	12.49	12.16	12.32	12.15	12.01
EE	3.00	3.10	3.15	3.10	3.30	3.15
Total Ash	7.28	7.20	7.01	7.00	6.64	6.78
NDF	56.40	53.50	55.10	56.75	55.80	56.76
ADF	28.35	27.45	29.85	28.80	29.00	30.30
Hemicellulose	28.05	26.05	25.25	27.95	26.80	26.46
ADL	5.05	4.70	5.20	5.15	5.10	5.20
TCHO	77.61	77.21	77.68	77.58	77.91	78.07

OM- Organic matter, CP- Crude protein, EE- Ether extract, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin, TCHO- Total carbohydrates.

Table 2: *In vitro* gas production and digestibility of TMRs containing graded levels of *C. tora* seed meal (24 h)

Parameter	TMR1	TMR2	TMR3	TMR4	TMR5	TMR6	SEM
NGP, ml/g DM/24h	193.33^{b}	194.00^{b}	196.00^{b}	200.67^{b}	184.00^{a}	182.67^{a}	1.99
PF, mg/ml	3.27^{a}	3.31^{a}	3.28^{a}	3.27^{a}	3.56^{b}	3.64^{b}	0.05
OMD,%	68.22	69.25	69.17	70.46	70.15	71.25	0.34
NDFD, %	47.75	48.67	47.97	51.60	50.06	52.78	0.63
MMP, mg	77.70^{a}	80.97^{a}	79.52^{a}	80.19^{a}	93.82^{b}	98.39^{b}	2.49
EMMP, %	32.75^{a}	33.58^{a}	32.96^{a}	32.63^{a}	38.20^{b}	39.50^{b}	0.87
DMD, %	69.07	70.40	70.00	71.73	70.93	72.00	0.34
SCFA, mmole	0.85^{b}	0.86^{b}	0.87^{b}	0.89^{b}	0.81^{a}	0.81^{a}	0.01
ME, MJ/kg DM	8.22^{bc}	8.30^{cd}	8.36^{cd}	8.51^{d}	8.05^{ab}	7.95^{a}	0.06
NH_3-N, mg/dl	24.23^{b}	24.51^{b}	22.67^{ab}	21.82^{ab}	19.84^{a}	19.98^{a}	0.60

NGP- Net gas production, PF- Partitioning factor, D- Digestibility, OM- Organic matter, NDF- Neutral detergent fibre, MMP- Microbial mass production, EMMP- Efficiency of microbial mass production, DM- Dry matter, SCFA- Short chain fatty acids, NH_3-N-Ammoniacal nitrogen, Means bearing different superscripts in a row differ significantly (P<0.05).

The partitioning factor (PF; mg/ml) was similar in TMR 1 (3.27), TMR 2 (3.31), TMR 3 (3.28) and TMR 4 (3.27), while significantly higher (P<0.05) PF values were recorded in TMR 5 (3.56) and TMR 6 (3.64) than other TMRs, indicating greater incorporation of degraded substrate into microbial biomass in these rations.

The OM digestibility (%) showed no significant difference (P>0.05) among the TMRs and was 68.22, 69.25, 69.17, 70.46, 70.15 and 71.25 % in TMR 1, TMR 2, TMR 3, TMR 4, TMR 5 and TMR 6, respectively. In contrast to the current study, Antonius *et al*. (2024) reported that supplementation of *Cassia alata* (CSA) significantly improved (P<0.05) *in vitro* OM digestibility of a palm kernel meal based ration, with 30% CSA inclusion yielding the highest IVOMD (61.6±4.26 %) compared with the control (31.3±1.22 %). Wahyuni *et al*. (2024) also demonstrated that supplementation of a corn stover based TMR with *Cassia* species (*Cassia siamea, C. fistula, C. surattensis, and C. spectabilis*) leaf extracts significantly improved (P<0.001) *in vitro* OM digestibility. However, Akinbode *et al*. (2023) reported that increasing inclusion of *Cassia fistula* leaf powder significantly and linearly reduced (P<0.001) *in vitro* OM digestibility.

The NDF digestibility (%) did not differ significantly (P>0.05) among TMRs and was 47.75, 48.67, 47.97, 51.60, 50.06 and 52.78 in the respective TMRs. The microbial mass production (MMP) varied significantly (P<0.05) among the TMRs tested. MMP (mg) was similar in TMR 1 (77.70), TMR 2 (80.97), TMR 3 (79.52) and TMR 4 (80.19), whereas MMP was significantly higher (P<0.05) in TMR 5 (93.82) and TMR 6 (98.39) than other TMRs tested. A similar trend was observed in the efficiency of microbial mass production (EMMP; %), with comparable values of 32.75, 33.58, 32.96 and 32.63 in TMR 1, TMR 2, TMR 3 and TMR 4, respectively, and significantly higher (P<0.05) values in TMR 5 (38.20) and TMR 6 (39.50) than other TMRs tested.

The DM digestibility did not vary significantly (P>0.05) among the TMRs. The results of the current study are in tune with those of Vansola *et al*. (2025) who reported that graded inclusion of *Senna gardneri* (leguminous plant related to genus *Cassia*) dry leaves at graded levels (1, 2, 3, 4, 5, 6, 7 and 8 %) in TMR did not significantly (P>0.05) affect *in vitro* DM digestibility. In contrary, Antonius *et al*. (2024) reported that 30% *Cassia alata* supplementation significantly increased (P<0.05) *in vitro* DM digestibility, with IVDMD rising from 41.2±1.71 % in the control to 70.5±4.52 %, indicating markedly improved ruminal DM utilization. Wahyuni *et al*. (2024) also showed that supplementation of a corn stover based TMR with *Cassia* species (*Cassia siamea, C. fistula, C. surattensis, and C. spectabilis*) leaf extracts significantly increased (P<0.001) *in vitro* DM digestibility. However, Akinbode *et al*. (2023) revealed that increasing inclusion of *Cassia fistula* leaf powder significantly reduced [linear (P<0.001); quadratic effects (P=0.018)] the *in vitro* DM digestibility, declining from 0.62 in the control to 0.44 at 60 mg/g DM (CF60).

The production of short-chain fatty acids (SCFA) differed significantly (P<0.05) among the TMRs. TMR 1, TMR 2, TMR 3 and TMR 4 recorded higher(P<0.05) SCFA values of 0.85, 0.86, 0.87 and 0.89, respectively, whereas significantly lower (P<0.05) SCFA production was observed in TMR 5 (0.81) and TMR 6 (0.81). Our results are similar to those of Akinbode *et al*. (2023) who reported that increasing inclusion of *Cassia fistula* leaf powder (CFLP) significantly reduced (P=0.002) total VFA production, with a linear decline (P<0.001) from 84.3 mM in the control to 75.3 mM at 60 mg/g DM. This reduction in TVFA indicated suppressed ruminal fermentative activity at higher CFLP inclusion levels. Wahyuni *et al*. (2024) reported that *Cassia* species (*Cassia siamea, C. fistula, C. surattensis, and C. spectabilis*) leaf extracts (2 mg/ml) significantly (P<0.001) altered total VFA concentration, 66.1 mM in T1 (*C. siamea* extract), 74.0 mM in T2 (*C. fistula* extract), 79.4 mM in T3 (*C. surattensis* extract) and 80.7 mM in T4(*C. spectabilis* extract) compared with 77.9 mM in the control. However, Antonius *et al*. (2024) reported that inclusion of *Cassia alata* at 30 % in a palm kernel meal based ration did not significantly (P>0.05) affect total volatile fatty acid (TVFA) concentration.

The metabolisable energy (ME) content also differed significantly (P<0.05) among the TMRs. The highest (P<0.05) ME value (MJ/kg DM) was recorded in TMR 4 (8.51), which was comparable to TMR 2 (8.30) and TMR 3 (8.36), while significantly lower (P<0.05) ME values were observed in TMR 5 (8.05) and TMR 6 (7.95) than other TMRs tested. The results of the current study are consistent with those of Akinbode *et al*. (2023) who observed a significant decline (P=0.002; linear P<0.001) in ME availability with increasing inclusion of *Cassia fistula* leaf powder (CFLP), with ME values progressively decreasing from 9.8 MJ/kg DM in the control to 8.1 MJ/kg DM at the highest inclusion level (CF 60: 60 mg/g DM).

The ammoniacal nitrogen (NH_3-N) production differed significantly (P<0.05) among the TMRs tested. Higher (P<0.05) NH_3-N values (mg/dl) were recorded in TMR 1 (24.23) and TMR 2 (24.51), with intermediate values in TMR 3 (22.67) and TMR 4 (21.82), and significantly lower (P<0.05) concentrations in TMR 5 (19.84) and TMR 6 (19.98). The results are similar to those of Akinbode *et al*. (2023) who reported that increasing inclusion level of *Cassia fistula* leaf powder (CFLP) significantly reduced (P=0.010; linear P=0.001) *in vitro* NH_3-N concentration, from 36 mg/dl in the control to 19 mg/dl at 60 mg/g DM inclusion level. The progressive reduction in NH_3-N indicated effective suppression of ruminal ammonia formation at higher CFLP inclusion levels in their study. In contrary to results of current study, Antonius *et al*.(2024) reported that *Cassia alata* (CSA) supplementation significantly increased (P<0.05) *in vitro* ruminal NH_3-N concentration, in the 30% CSA diet. Diets containing *C. alata*, either alone or in combination, consistently showed higher NH_3-N levels (≈16.0–16.7 mM), indicating enhanced ruminal nitrogen availability. Wahyuni *et al*. (2024) reported that supplementation of corn stover based TMR with *Cassia* species (*Cassia siamea, C. fistula, C. surattensis, and C. spectabilis*) leaf extracts significantly (P=0.008) affected *in vitro* NH_3-N concentration, with higher values observed for *C. surattensis* and *C. spectabilis* compared with the control. Aragadvay-Yungán *et al*. (2021) reported that *in vitro* ruminal NH_3-N concentration did not differ (P=0.1291) among tropical forage legumes (*Clitoria arborea, Erythrina fusca, Bauhinia forficata, Erytrina poeppigiana, Cratylia argentea, Glirycidia sepium, Cassia tora,* and *Flemingia macrophylla*) after 24 h incubation, with values ranging from 109.45 to 220.35 mg/l. *Cassia tora* showed an intermediate NH_3-N production (142.83 mg/l), indicating no marked effect on ruminal ammoniacal nitrogen availability intheir study.

CONCLUSION

In vitro evaluation of TMRs indicated that inclusion of graded levels of *Cassia tora* seed meal did not affect (P>0.05) nutrient digestibility (OM, NDF, DM) across treatment groups. However, TMRs containing higher inclusion levels (10% and 12% in the concentrate mixture) exhibited a significant reduction (P<0.05) in SCFA production and ME availability, suggesting reduced fermentation efficiency, beyond 8% inclusion level. Considering the combined effects on rumen fermentation and energy supply, it was concluded that *Cassia tora* seed meal could be incorporated upto 8% level (w/w basis) in the concentrate mixture without affecting nutrient digestibility, SCFA production and ME availability.

GENERATIVE AI DECLARATION

The author(s) confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the

writing or editing of the manuscript, and no images were manipulated using AI. The authors developed and verified all scientific content, data analysis, interpretation of results, and conclusions. The authors take full responsibility for the accuracy, integrity, and originality of the work presented, and no AI tool was listed as an author.

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