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Short Communication

In vitro assessment of rumen fermentation using diverse total mixed rations supplemented with bee propolis: A comparative study

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Abstract

The present study aimed to compare *in vitro* total mixed rations (TMRs) and to assess the effect of bee propolis as a potential feed additive on rumen fermentation parameters. Four TMRs with R: C ratio of 65:35 were prepared and designated as TMR 1 (maize silage-based), TMR 2 (maize fodder-based), TMR 3 (oat silage-based) and TMR 4 (napier bajra fodder-based). Bee propolis was added to all the TMRs at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% on DM basis, each of which was used as a substrate. The fermentation parameters of TMRs, irrespective of the level of bee propolis, revealed that net gas production (NGP) varied significantly (P≤0.05) among different TMRs, with the highest (P≤0.05) NGP in maize fodder-based TMR 2, followed by maize silage-based TMR 1. The digestibility of organic matter (OM), dry matter (DM), short chain fatty acids (SCFA) production and metabolizable energy (ME) availability were higher (P≤0.05) in TMRs containing maize fodder and maize silage. The fermentation pattern of TMRs (irrespective of the fodder used) revealed that NGP, digestibility of nutrients (OM, DM, NDF), microbial mass production (MMP) and efficiency of MMP (EMMP) were similar across the various selected levels of bee propolis. However, NH3-N (ammoniacal nitrogen) production decreased (P≤0.05) in the *in vitro* medium (buffalo rumen liquor) at 0.2% (DM basis) inclusion level of bee propolis. It was concluded that bee propolis could be added at a rate of 0.2% (DM basis) to the TMR of ruminants as it has the potential to decrease ammonia-N production.

Keywords: Bee propolis, *In vitro*, Maize, Napier bajra, Oats

Highlights

- NGP was highest (P≤0.05) in maize (fodder and silage) based TMRs, irrespective of propolis level.
- *In vitro* OM and DM digestibility was higher (P≤0.05) in maize based TMRs than other TMRs.
- SCFA production and ME availability were also higher (P≤0.05) in maize based TMRs than other TMRs.
- Bee propolis supplementation at 0.2% (DM basis) lowered (P≤0.05) *in vitro* NH_3-N production.

Adequate nutrition forms the basis of sustainable livestock production systems. Among the factors affecting livestock performance, fodder quality and digestibility significantly affect nutrient intake and utilization (Bashar et al., 2024). Comparative *in vitro* studies are important for discovering high-quality forages and learning how various diets function under standardized settings (Ortiz & Vega, 2020). Besides forage quality, feed additives have emerged as a means to improve ruminal fermentation and nutrient use. Concerns about antimicrobial resistance and the phase-out of antibiotic growth promoters have driven interest in natural alternatives (Hassan et al., 2020). Moreover, synthetic chemicals leave residues in animal-based food products like meat and milk, ultimately affecting human beings.

One such natural feed additive is bee propolis, also known as bee glue. Bee propolis is a sticky substance produced by honeybees (*Apis mellifera*) by combining resinous substances collected from plant sources with their salivary secretions. Propolis is used by bees to build their hives and protect themselves from pathogens and predators (Abduh et al., 2024). Bee propolis is known to have over 800 chemical compounds like chalcones, benzofurans, benzopyranes, flavonoids and their esters, acids, alcohols and their esters, glycosides (flavonoid and diterpene), which attribute to its wide range of biological properties like antimicrobial, antioxidant, anticancer, immunomodulatory and anti-inflammatory activities (Kasote et al., 2022).Therefore, the present study aimed at a comparative *in-vitro* evaluation of various TMRs based on different fodders/silages and to study the effect of bee propolis as a potential natural feed additive on rumen fermentation parameters.

Four TMRs with a roughage: concentrate ratio of

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65:35 was prepared and designated as TMR 1 (maize silage-based), TMR 2 (maize fodder-based), TMR 3 (oat silage-based) and TMR 4 (napier bajra fodder-based). The roughage portion was made up of wheat straw and fodder/ silage in a 50:50 ratio. The conventional concentrate was made up of maize 35%, soybean meal 15%, mustard cake 15%, wheat bran 9%, rice polish 7%, deoiled rice bran 15.5%, mineral mixture 2%, salt 1% and urea 0.5% each. The finely ground complete feeds were analyzed for proximate principles (Association of Official Analytical Chemists [AOAC], 2007) and cell wall constituents (Robertson & Van Soest, 1991). Bee propolis was procured from local market and added to all the TMRs at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% on DM basis, each, of which was used as substrates. *In vitro* evaluation was done by *in vitro* gas production technique (Menke & Steingass, 1988).

Rumen liquor was collected from rumen fistulated male buffaloes fed a 2 kg conventional concentrate mixture, 8 kg green fodder, 1 kg wheat straw, and *ad libitum* urea molasses mineral block. The rumen contents were strained over 4-ply muslin cloth. CO_2 was flushed while the reducing solution was added. The strained rumen liquor (SRL) was added in a 1:2 ratio to the buffer media. Each syringe contained 375 mg test sample and received 30 mL of SRL: buffer solution from the flask held in a water bath. The clip was quickly closed, and the exact volume of the contents in the syringe was recorded and stored in a water bath at 39°C. For the first few hours, the contents of all the syringes were swirled at 1-hour intervals. If the gas volume exceeded 70 mL after 8 hours, the volume was recorded and the gas was removed. The volume of gas produced in each syringe was measured after 24 hours. The data were analyzed in simple ANOVA, as described by Snedecor and Cochran (1994), by using SPSS (2012) version 21; and the mean differences were tested using Tukey's b test.

Bee propolis used in the comparative *in vitro* study consisted of organic matter 95.63%, total ash 4.37%, crude protein 2.51%, ether extract 1.10%, crude fibre 0.60% and total carbohydrates 92.02% on DM basis.

The chemical composition of TMRs used in the *in vitro* analysis is given in Table 1. The crude protein content in the TMRs ranged from 11.71% to 12.72%. The ether extract in the TMRs ranged from 2.73% to 3.05%.

The fermentation parameters of TMRs, irrespective of the level of bee propolis, are presented in Table 2. The net gas production (NGP) varied significantly ($P \leq 0.05$) among different TMRs, with the highest ($P \leq 0.05$) NGP in maize fodder based TMR 2, followed by maize silage-based TMR 1 and the lowest ($P \leq 0.05$) NGP in both oat silage-based TMR 3 as well as napier bajra fodder-based TMR 4. The partitioning factor (PF) also differed significantly ($P \leq 0.05$) amongst TMRs, with the lowest ($P \leq 0.05$) value in TMR 2, followed by TMR 1, TMR 4 and the highest ($P \leq 0.05$) value in TMR 3. The OM, NDF and DM digestibility varied significantly ($P \leq 0.05$) among the various TMRs. The Organic matter digestibility (OMD) was similar in TMR 1 (maize silage), 2 (maize fodder) and 3 (oat silage), with the lowest ($P \leq 0.05$) value in napier bajra fodder-based TMR 4. Further, in the present study, NDFD was lowest ($P \leq 0.05$) in maize silage-based TMR 1, followed by maize fodder-based TMR 2 and napier bajra fodder-based TMR 4, with the highest ($P \leq 0.05$) value in oat silage-based TMR 3. Moreover, in the current study, DM digestibility also showed a significant difference among TMRs, with TMR 1 (maize silage) and 2 (maize fodder) having the highest ($P \leq 0.05$) values, followed by TMR 3, and the lowest ($P \leq 0.05$) value in TMR 4.

Microbial mass production (MMP) was highest ($P \leq 0.05$) in TMR 3, followed by TMR 4 and TMR 1, and was lowest ($P \leq 0.05$) in TMR 2. The efficiency of microbial mass production (EMMP) was highest

Table 1. Chemical composition of various TMRs used in the *in vitro* experiment (% DM basis)

Parameter	TMR 1 (Maize silage)	TMR 2 (Maize fodder)	TMR 3 (Oat silage)	TMR 4 (Napier bajra fodder)
Organic matter	92.42	93.12	90.52	91.57
Total ash	7.58	6.88	9.48	8.43
Crude protein	12.16	12.40	12.72	11.71
Ether extract	3.05	2.85	3.00	2.73
Neutral detergent fibre	55.70	56.90	69.20	64.90
Acid detergent fibre	35.60	31.15	36.90	36.35
Hemicellulose	20.10	25.75	32.30	28.55
Cellulose	22.80	21.70	25.40	25.50
Acid detergent lignin	5.30	4.35	5.10	5.50
Total carbohydrates	77.21	77.87	74.80	77.13

Table 2. Fermentation parameters of TMRs, irrespective of level of bee propolis used

Parameter	TMR1 (Maize silage)	TMR2 (Maize fodder)	TMR 3 (Oatssilage)	TMR4 (Napier bajra fodder)	SEM
NGP, mL/g DM/ 24 h	155.44^{b}	171.33^{c}	132.42^{a}	135.33^{a}	2.36
PF, mg/mL	3.75^{b}	3.43^{a}	4.32^{d}	3.98^{c}	0.05
OMD, %	63.04^{b}	63.03^{b}	63.08^{b}	58.84^{a}	0.35
NDFD, %	38.68^{a}	39.50^{ab}	51.71^{c}	41.93^{b}	0.84
MMP, mg	90.24^{b}	78.76^{a}	104.91^{c}	90.39^{b}	1.57
EMMP, %	41.29^{b}	35.73^{a}	48.98^{d}	44.69 ^{c}	0.75
DMD, %	67.29^{c}	67.09^{c}	63.47^{b}	60.96^{a}	0.46
SCFA, mmole	0.69^{b}	0.76^{c}	0.58^{a}	0.60 ^{a}	0.01
ME, MJ/kg DM	7.37^{b}	7.74^{c}	6.73^{a}	6.68 ^{a}	0.07
NH_3-N, mg/dL	22.05^{b}	20.63^{ab}	21.57^{b}	19.90 ^{a}	0.24

TMR 1- Maize silage based, TMR 2- Maize fodder based, TMR 3- Oat silage based, TMR 4- Napier bajra fodder based, 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, ME- Metabolizable energy, NH_3-N- Ammoniacal nitrogen, R:C ratio was 65:35 on dry matter basis, Means bearing different superscripts in a row differ significantly (P≤0.05).

Table 3. Fermentation parameters of TMRs, irrespective of fodder used in TMRs

	Level of bee propolis (% DM basis)						
Parameter	**0**	**0.025**	**0.05**	**0.1**	**0.15**	**0.2**	**SEM**
NGP, mL/g DM/24 h	148.12	146.33	146.50	149.33	149.83	151.67	2.36
PF, mg/mL	3.90	3.90	3.96	3.84	3.82	3.80	0.05
OMD, %	62.42	61.41	62.43	61.74	62.34	61.66	0.35
NDFD, %	43.77	41.99	43.43	42.47	43.60	42.48	0.84
MMP, mg	93.02	90.92	94.42	89.57	90.32	88.22	1.57
EMMP, %	43.29	42.98	43.90	42.13	42.13	41.59	0.75
DMD, %	64.85	64.40	65.30	64.77	65.03	63.87	0.46
SCFA, mmole	0.65	0.65	0.65	0.66	0.67	0.66	0.01
ME, MJ/kg DM	7.12	7.06	7.06	7.15	7.20	7.19	0.07
NH_3-N, mg/dL	21.35^{b}	21.00^{ab}	21.76^{b}	21.43^{b}	21.48^{b}	19.19^{a}	0.24

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, ME- Metabolizable energy, NH_3-N- Ammoniacal nitrogen, R:C ratio was 65:35 on dry matter basis. Means bearing different superscripts in a row differ significantly (P≤0.05).

(P≤0.05) in TMR 3, followed by TMR 4 and TMR 1, and lowest (P≤0.05) in TMR 2 (Table 2). Further, production of short chain fatty acids (SCFA) was highest (P≤0.05) in TMR 2 (maize fodder), followed by TMR 1 (maize silage) and lowest (P≤0.05) in TMR 3 (oats silage) and TMR 4 (napier bajra). ME availability had the highest (P≤0.05) value in maize fodder-based TMR. ME availability showed a trend similar to SCFA production, with highest (P≤0.05) value in TMR 2 (maize fodder). *In vitro* NH3-N production varied significantly (P≤0.05) among TMRs tested, with the lowest (P≤0.05) value in napier bajra-based TMR 4.

The fermentation parameters of TMRs, irrespective of the fodder used, are presented in Table 3. The NGP, PF, and digestibility of nutrients (OM, NDF, DM) varied non-significantly (P≤0.05) across different levels of bee propolis. The partitioning factor is defined as the ratio of organic matter degraded (mg) *in vitro* to the volume of gas (mL) produced. A higher PF means that proportionally more of the degraded matter is incorporated into microbial mass, i.e. the efficiency of microbial protein synthesis is higher. The PF of ruminant diets should lie in the range of 2.71 to 4.41 (Blümmel et al., 1997). The PF in the present study ranged from 3.80 to 3.96, which is within the suggested range.

Microbial mass production, EMMP, SCFA and availability of ME were also similar across various levels of bee propolis tested. However, the ammonia

nitrogen (NH_3-N) levels decreased (P≤0.05) at 0.2% inclusion level of bee propolis. Decrease in rumen NH_3-N levels in ruminants suggests better synchronization between energy and nitrogen supply in the rumen, improved nitrogen utilization efficiency with more efficient incorporation of nitrogen into microbial protein and reduced nitrogen losses, which is economically and environmentally favourable. It reflects an optimal balance between protein degradation and microbial nitrogen demand, enhancing microbial growth and activity. Therefore, lower levels of rumen NH_3-N offer the benefits of improved feed efficiency and potentially better animal health.

Therefore, the study concluded that bee propolis could be added at a rate of 0.2% (DM basis) to the TMR of ruminants as it has the potential to decrease ammonia-N production and to reduce the impact of ruminant production systems on the environment. *In vitro* OM and DM digestibility was higher (P≤0.05) in maize based TMRs than other TMRs and SCFA production as well as ME availability were higher (P≤0.05) in maize based TMRs than other TMRs.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: SD: Performed lab analysis, collection of data, drafting manuscript; JK: Planned the study, performed statistical analysis of data. RSG: Offered continuous guidance throughout the study and contributed to the overall quality of the research. The final manuscript was read and approved by all authors.

Data availability statement: Data is contained within the article. Data of research conducted is preserved by the author and will be made available upon demand.

Ethical approval: This study was conducted after an approval from "Institutional Animal Ethical Committee (IAEC)" on May 24, 2024, under protocol number IAEC/73/01/2024.

Use of artificial intelligence tools: No artificial intelligence tools were used.

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