Journal of Animal Research: v.15 n.04, p. 133-140. August 2025

**DOI:** 10.30954/2277-940X.04.2025.3

# Maize Silage Based Total Mixed Ration and Bee Propolis: An *In Vitro* Approach to Rumen Fermentation Dynamics

**Srishti Dhuria, Jasmine Kaur*, R.S. Grewal and J.S. Lamba**

*Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, INDIA*

**Corresponding author: J Kaur; E-mail: sachdeva_jasmine@rediffmail.com*

**Received:** 12 June, 2025 **Revised:** 12 July, 2025 **Accepted:** 16 July, 2025

**ABSTRACT**

The current study was designed to evaluate the chemical composition and *in vitro* nutritional worth of maize silage based total mixed ration (R:C ratio of 65:35) consisting of graded levels of bee propolis. Bee propolis was added to maize silage based total mixed ration (TMR) at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% on DM basis. The net gas production, partitioning factor, digestibility of nutrients (OM, NDF, DM), microbial mass production, efficiency of microbial mass production, short chain fatty acids and metabolizable energy (ME) were not affected by the addition of graded levels of bee propolis. However, inclusion of bee propolis @ 0.2 percent (DM basis) significantly reduced (P≤0.05) ammoniacal–N production ($NH_3$-N) in the *in vitro* medium. Therefore, bee propolis could be added at 0.2% level on DM basis in the maize silage based TMR without affecting feed digestibility and microbial mass production with a potential to decrease nitrogen losses and improve feed efficiency.

**HIGHLIGHTS**

- Effects of adding graded levels of bee propolis to maize silage based TMR were studied *in vitro*.
- *In vitro* ammonia-N decreased ((P≤0.05) at 0.2% inclusion level of bee propolis in TMR.

**Keywords:** Ammoniacal nitrogen, Bee propolis, *In vitro* evaluation

India has the largest livestock population in the world with around 536.76 million animals (DAHD, 2024). The importance of livestock farming in India's agricultural sector is increasing as the demand for products derived from livestock, such as milk and meat, grows in tune with the growing focus on natural products and organic farming. The demand for organic and natural alternatives has increased as a result of restrictions placed by some countries on the use of synthetic drugs and antibiotics in the livestock sector due to growing worries about antibiotic residues in food. The growing public demand for food items devoid of antibiotics has led researchers to investigate novel, organic additives for feed that have advantages similar to those of antibiotics. The bee propolis produced by honey bees is one such promising alternative.

With growing interest in organic food products as well as conservation of honey bees, bee products have gained the limelight. Moreover, bee products have higher bioavailability as compared to artificially produced preparations (Madras-Majewska *et al*., 2015). Bee propois is also known as 'bee glue'. The term 'propolis' comes from two greek words- 'pro' and 'polis', which refer to 'in front' and 'city', respectively, which highlights the protective role of propolis in a bee colony as a first line of defense against invaders like snakes, lizards, wind and rain etc. Propolis is a resinous substance produced by honey bees (*Apis mellifera*) by collecting substances from cracks in the bark, leaves and plant secretions and mixing them with pollen, wax, honey and their salivary enzymes.

Bee propolis has a wide spectrum of biological properties like antibacterial, antiviral, antifungal, anticarcinogenic,

**How to cite this article:** Dhuria, S., Kaur, J., Grewal, R.S. and Lamba, J.S. (2025). Maize Silage Based Total Mixed Ration and Bee Propolis: An *In Vitro* Approach to Rumen Fermentation Dynamics. *J. Anim. Res.,* **15**(04): 133-140.

**Source of Support:** None; **Conflict of Interest:** None

anti-inflammatory, antibiotic, antioxidative, anesthetic, antiparasitic and immunostimulatory effects (Wagh, 2013). Due to this extensive array of beneficial properties, bee propolis is being extensively employed in cosmetics, pharmacology, human and veterinary medicine. Moreover, bee propolis is an environment friendly, biodegradable, organic product that does not leave harmful chemical residues in the livestock products (milk and meat) like antibiotics.

In view of such beneficial properties of bee propolis, the present *in vitro* experiment was conducted to study its effect on fermentation parameters in terms of net gas production, partitioning factor, digestibility of nutrients (OM, DM and NDF), microbial mass production, efficiency of microbial mass production, short chain fatty acid production, metabolizable energy and ammoniacal nitrogen.

## MATERIALS AND METHODS

The present study was conducted in the Department of Animal Nutrition, GADVASU. The research was approved by the Institutional Animal Ethics Committee. The bee propolis was procured from local market.

### Experimental diets and treatments

TMR had a roughage to concentrate ratio of 65:35 and the roughage portion was made up of wheat straw and maize silage in 50:50 ratio (Table 2). Bee propolis was added to TMR at graded levels of 0, 0.025, 0.05, 0.1, 0.15 and 0.2% (DM basis).

### Chemical analysis

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

### *In vitro* evaluation

Rumen liquor was procured from fistulated male buffaloes that were fed 2 kg of conventional concentrate, 15 kg of fresh green forage and 3 kg of wheat straw. After being collected in a thermos flask, the contents of the rumen were kept at 39°C and flushed with $CO_2$. The contents of the rumen were processed in a blender for two to three minutes while the temperature was maintained at 39°C. The mixture was then strained through four-ply muslin fabric. While the reducing solution was being added, $CO_2$ was flushed via a submerged tube. A slight bluish colour changed to pink and ultimately turned colourless in appearance. Only when the solution turned colourless, the strained rumen liquor (SRL) was added to the buffer medium (consisting of micro, macro mineral solutions, resazurin and a bicarbonate buffer solution (Menke *et al*., 1979; Menke and Steingass, 1988) in a 1:2 ratio. Continuous $CO_2$ flushing was performed until the final syringe was filled. The tube on the syringe's capillary connection was securely fastened to the bottle top dispenser in order to fill syringes. Each syringe received a pump of 30 ml of SRL- buffer solution from the flask placed in water bath. The syringe was gently shaken for mixing its contents. By carefully moving the piston upward, air bubbles were brought to the surface and then expelled via the capillary. The clip was closed and the precise volume of the contents of the syringe was recorded. The syringe was then placed in a water bath adjusted at 39°C. For the initial couple of hours, the contents of each syringe were swirled once per hour. If the gas surpassed 70 ml after 8 hours, it was expelled after measuring the volume of gas. After 24 hours, the amount of gas generated in each syringe was measured. With every incubation set, blank and standard hay were also run in triplicates. The residue's $NH_3$-N, and NDF were measured after 24 hours. ME was computed using the volume of gas generated (Menke *et al*., 1979). The partitioning factor (PF) was estimated as the ratio of substrate actually degraded *in vitro* (mg) to the volume of gas generated (ml) by it (France *et al*., 1993).

### Statistical analysis

The data generated during the study were analysed with the help of (SPSS, 2012) version 21 using simple ANOVA. The differences in the means were tested by Tukey's b.

## RESULTS AND DISCUSSION

### Chemical composition of bee propolis

The chemical composition of bee propolis used in this study is presented in Table 1. Bee propolis contained 95.63% organic matter and 4.37% total ash. Pant *et al*.

(2021) reported that ash percentage in north India's propolis samples varied significantly from 3.01%- 4.71%, which is similar to our result. However, Fallah *et al*. (2021) evaluated propolis samples and reported ash content of 2.76±0.22 g/100g in Iranian propolis samples. Sierra-Galicia *et al*. (2022) found the ash content of Mexican propolis samples to be 0.85%.

The bee propolis used in present study contained 2.51% crude protein. Our finding was similar to that reported by Sierra-Galicia *et al*. (2022) who collected propolis samples from Mexico and reported 2.55% of crude protein content. However, Fallah *et al*. (2021) reported 11.00±0.85 g/100g protein in Iranian propolis samples. Pant *et al*. (2021) found that crude protein concentration ranged from 7.28% to 9.41% in the north Indian propolis samples.

The bee propolis used in present study contained 1.10% ether extract, which was lower than that reported by Sierra-Galicia *et al*. (2022) (9.31%). However, Pant *et al*. (2021) reported that crude fat percentage ranged between 53.62% and 68.89% in north Indian propolis samples.

The bee propolis used in present study contained 0.60% crude fibre. Pant *et al*. (2021) reported that crude fibre ranged from 1.94% to 3.15% in north Indian propolis samples. Total carbohydrates content of bee propolis used in the present study is 92.02%.

**Table 1:** Chemical composition of bee propolis, % DM basis

| Parameters | Bee propolis |
|---|---|
| OM | 95.63 |
| CP | 2.51 |
| EE | 1.10 |
| Total ash | 4.37 |
| Crude fibre | 0.60 |
| TCHO | 92.02 |

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

## Ingredient and chemical composition of the maize silage based TMR

The ingredient and chemical composition of the maize silage based TMR is presented in Table 2 and 3, respectively. In the TMR, the roughage portion was made up of wheat straw and maize silage in 50:50 ratio. The concentrate ingredients used in the TMR comprised of maize 12.25 %, soybean meal 5.25%, mustard cake 5.25%, wheat bran 3.15%, rice polish 2.45%, deoiled rice bran 5.425%, mineral mixture 0.7%, common salt 0.35% and urea 0.175%. The chemical constituents of the TMR contained organic matter 92.42%, total ash 7.58%, crude protein 12.16%, ether extract 3.05%, neutral detergent fibre 55.70%, acid detergent fibre 35.60%, hemicellulose 20.10%, cellulose 22.80%, acid detergent lignin 5.30% and total carbohydrates 77.21%.

**Table 2:** Ingredient composition of maize silage based TMR used in the *in vitro* experiment (% DM basis)

| Ingredient | Percent |
|---|---|
| Wheat straw | 32.5 |
| Maize silage | 32.5 |
| **Concentrate Ingredients** | |
| Maize | 12.25 |
| Soybean meal | 5.25 |
| Mustard cake | 5.25 |
| Wheat Bran | 3.15 |
| Rice polish | 2.45 |
| Deoiled rice bran | 5.425 |
| Mineral mixture | 0.7 |
| Common salt | 0.35 |
| Urea | 0.175 |

**Table 3:** Chemical composition of maize silage based TMR used in the *in vitro* experiment (% DM basis)

| Parameter | Percent |
|---|---|
| Organic matter | 92.42 |
| Total ash | 7.58 |
| Crude protein | 12.16 |
| Ether extract | 3.05 |
| Neutral detergent fibre | 55.70 |
| Acid detergent fibre | 35.60 |
| Hemicellulose | 20.10 |
| Cellulose | 22.80 |
| Acid detergent lignin | 5.30 |
| Total carbohydrates | 77.21 |

***In vitro* evaluation of maize silage based TMR containing graded levels of bee propolis**

The fermentation parameters of TMR (maize silage based TMR) are presented in Table 4. The net gas production (NGP) in maize silage based TMR was found to be 157.33, 152.67, 150.67, 156.67, 157.33 and 158.00 ml/g DM/24h at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% levels of bee propolis, respectively (Table 4).There was no significant difference in NGP among the various levels of bee propolis included in TMR. Our results are in line with those of Nascimento *et al*. (2020), who studied the impact of including propolis extraction residue (100 g/cow/day) into bovine diets fed silage alongwith varying concentrate levels (25, 50 and 75%) on *in vitro* ruminal fermentation and reported that addition of propolis into bovine diets had no significant effect on total volume of gases produced in the treatment (25, 50, and 75%) and control group (without propolis extraction residue). Further, Morsy *et al*. (2015) used a semi-automatic *in vitro* gas production system to compare the effects of increasing levels of ethanolic extracts of Brazilian red propolis and Egyptian brown propolis on ruminal degradation of nutrients and methane production, and found no significant differences in net gas production at various propolis levels (0, 125, 250, and 500µg per 500 mg of dietary dry matter). Moreover, Morsy *et al*. (2011) reported no significant (P>0.05) variation in gas production among both Brazilian propolis extracts, Brazilian green propolis (BGP) and Brazilian alamo propolis (BAP) at various doses (125, 250, and 500 µg/75 ml culture fluid) as well as in control. However, in an *in vitro* study by Coşkuntuna *et al*. (2023), who studied the effects of adding 0.05% propolis extract to sorghum grain varieties (Es8z102, Albanus, Sugar Drip, Gül Şeker and Csr9303) containing different levels of tannins on *in vitro* gas production parameters (measured after 24, 48, 72, and 96 hours of incubation), reported that gas production increased significantly ($P = 0.000$) in response to addition of propolis extract to all the sorghum grain varieties as compared to the control (without addition of propolis extract). The highest ($P = 0.000$) *in vitro* gas production was reported in the Csr9303 variety of sorghum having the lowest tannin content.

In the current study, the partitioning factor (PF, mg/ml) at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% levels of bee propolis was 3.79, 3.77, 3.82, 3.68, 3.71 and 3.72, respectively. No significant difference was seen in PF among graded levels of bee propolis in TMR. Our findings are consistent with those of Morsy *et al*. (2015), who used a semi-automatic *in vitro* gas production system to compare the effects of increasing levels of ethanolic extracts of Brazilian red propolis and Egyptian brown propolis on ruminal degradation of nutrients and methane production, and found no significant differences in PF among the various propolis levels (0, 125, 250, and 500µg per 500 mg of dietary dry matter). In addition, Morsy *et al*. (2011) reported no significant variation in PF among both Brazilian propolis extracts, Brazilian green propolis (BGP) and Brazilian alamo propolis (BAP) at various doses (125, 250, and 500 µg/75 ml culture fluid) as well as in control. The partitioning factor (PF) 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 calculated during *in vitro* provides useful information for predicting the dry matter intake (DMI), microbial mass production (MMP) in the rumen and methane ($CH_4$) emission of the entire ruminant animal. 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 3.68 to 3.82, which is within the suggested range.

There was no significant difference in the organic matter digestibility (OMD), NDF digestibility (NDFD) and DM digestibility (DMD) among various levels of bee propolis used in TMR (Table 4). The OMD (%) in TMR containing graded levels of bee propolis as 0, 0.025, 0.05, 0.1, 0.15 and 0.2 % was 64.51, 62.34, 62.20, 62.34, 63.21 and 63.64 %, respectively. The values of NDFD in TMR were 41.11, 37.52, 37.28, 37.52, 38.96, and 39.68% corresponding to the 0, 0.025, 0.05, 0.1, 0.15 and 0.2% levels of bee propolis, respectively. With inclusion of bee propolis at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% levels in TMR , the DMD was 68.13, 66.80, 66.67, 66.53, 67.87 and 67.73%, respectively. Our results are in line with those of Mahmood *et al*. (2022), who investigated the effects of different moringa by-products and raw propolis on ruminal fermentation using RUSITEC and reported that there was no significant (P>0.05) difference in the degradation of DM, OM and NDF across the treatments (control diet without supplementation and the control diet top-dressed with moringa seed cake, moringa leaf powder,

**Table 4:** Effect of level of bee propolis on the *in vitro* gas production and digestibility of nutrients in maize silage based TMR (24 h)

| Parameter | Level of bee propolis (% DM basis) | | | | | | SEM |
|---|---|---|---|---|---|---|---|
| | 0 | 0.025 | 0.05 | 0.1 | 0.15 | 0.2 | |
| NGP, ml/g DM/24h | 157.33 | 152.67 | 150.67 | 156.67 | 157.33 | 158.00 | 0.99 |
| PF, mg/ml | 3.79 | 3.77 | 3.82 | 3.68 | 3.71 | 3.72 | 0.02 |
| OMD, % | 64.51 | 62.34 | 62.20 | 62.34 | 63.21 | 63.64 | 0.43 |
| NDFD, % | 41.11 | 37.52 | 37.28 | 37.52 | 38.96 | 39.68 | 0.71 |
| MMP, mg | 93.76 | 90.11 | 91.26 | 86.81 | 89.26 | 90.21 | 1.30 |
| EMMP, % | 41.88 | 41.71 | 42.31 | 40.18 | 40.74 | 40.91 | 0.38 |
| DMD, % | 68.13 | 66.80 | 66.67 | 66.53 | 67.87 | 67.73 | 0.35 |
| SCFA, mmole | 0.69 | 0.67 | 0.66 | 0.69 | 0.69 | 0.70 | 0.00 |
| ME, MJ/ kg DM | 7.43 | 7.29 | 7.23 | 7.41 | 7.43 | 7.45 | 0.03 |
| $NH_3$-N, mg/dl | 22.51$^{b}$ | 23.13$^{b}$ | 24.29$^{b}$ | 22.85$^{b}$ | 21.74$^{b}$ | 17.79$^{a}$ | 0.64 |

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 ($P \leq 0.05$).

or raw propolis). Moreover, Nascimento *et al*. (2020), who studied the effect of including propolis extraction residue (100 g/cow/day) into bovine diets fed with silage alongwith varying concentrate levels (25, 50, and 75%) on *in vitro* ruminal fermentation and reported that addition of propolis into bovine diets had no significant effect on DMD in the treatment (25, 50, and 75%) and control group (without propolis extraction residue). Özturk *et al*. (2010) studied the effect of varying concentrations of propolis extract (0%, 20%, 60% propolis ethanolic extract) using RUSITEC and revealed that propolis supplementation had no significant effect on DM digestibility. However, Heimbach *et al*. (2014) assessed 5 different levels of incorporation of residue from propolis extraction as 0 (control), 5, 10, 15 and 20 g of residue/kg DM, for *in vitro* gas production and nutrient digestibility and reported a significant increase ($P<0.05$) in the *in vitro* ruminal (without addition of pepsin) and total (with addition of pepsin) digestibility of dry matter and neutral detergent fiber with increasing inclusion levels of residue from propolis extraction as compared to control.

Microbial mass production (MMP) did not vary significantly with increasing levels of bee propolis in TMR 1, with the MMP values as 93.76, 90.11, 91.26, 86.81, 89.26, and 90.21 mg at 0, 0.025, 0.05, 0.1, 0.15, and 0.2% levels of bee propolis, respectively (Table 4). Our findings are consistent with those of Morsy *et al*. (2021) who supplemented late-pregnant Santa Inês ewes with 3 g red propolis extract/ewe/day and reported that red propolis extract treatment had no significant ($P>0.05$) effect on amount of absorbed microbial protein and daily supply of microbial nitrogen as compared to control treatment (devoid of propolis supplementation). In the present study, efficiency of microbial mass production (EMMP) followed similar trend as MMP and there was no significant difference in the values of EMMP across various treatments. Our outcome corroborated with that of Valero *et al*. (2015), who examined the effect of substitution of monensin with propolis in the diet of feedlots bulls, and revealed that both microbial protein synthesis as well as the efficiency of microbial synthesis were unaffected ($P>0.05$) by the supplementation of monensin or propolis and were similar to control. Moreover, Aguiar *et al*. (2014) studied the effect of supplementation of three variations of propolis based products (PBP) as PBP B1, PBP C1 and PBP C3 (differing in the concentrations of phenolic compounds as 3.81 mg/kg, 3.27 mg/kg, and 1.93 mg/kg of ingested DM, respectively) to the diets of dairy cattle and reported that there was no significant ($P>0.05$) difference in the microbial protein synthesis and microbial efficiency on inclusion of propolis-based products (PBP B1, PBP C1 and PBP C3) in the diet as compared to control. Furthermore, De Paula *et al*. (2016) explored the implications of giving

propolis-derived phenolic compounds to water buffaloes in different doses (0, 16.95, 33.9, and 50.85 mg/d) and reported that there were no significant differences (P>0.05) in microbial protein synthesis and microbial protein synthesis efficiency among propolis supplemented groups and control.

The increasing dose of bee propolis in TMR resulted in non-significant changes in short chain fatty acids (SCFA), which were 0.69, 0.67, 0.66, 0.69, 0.69 and 0.70 mmole, corresponding to 0, 0.025, 0.05, 0.1, 0.15 and 0.2% levels of bee propolis in TMR, respectively. Our results corroborated with the results of Mahmood *et al*. (2022), who investigated the effects of different moringa by-products and raw propolis on ruminal fermentation using RUSITEC and found that dietary treatment with propolis had no significant (P>0.05) effect on total SCFA concentration as compared to control (devoid of propolis or moringa by-products). Moreover, Özturk *et al*. (2010), studied the effect of varying concentrations of propolis extract (0%, 20%, 60% propolis ethanolic extract) using RUSITEC and revealed that propolis supplementation had no significant effect on SCFA production. In the current study, metabolizable energy (ME) availability was 7.43, 7.29, 7.23, 7.41, 7.43 and 7.45 MJ/kg DM, corresponding to 0, 0.025, 0.05, 0.1, 0.15 and 0.2% levels of bee propolis, respectively and values were similar across various treatments. Our results are similar to the results recorded by Da Silva *et al*. (2025) who assessed the effect of supplementing increasing doses (0, 6, 12, 18, and 24 ml/day) of green propolis extract (GPE) in ration of feedlot rams for 85 days and found that metabolizable energy in the rams' diet was unaffected (P>0.05) by GPE supplementation across all treatment groups and was similar to control. Moreover, Mahmood *et al*. (2022) studied the effects of different moringa by-products and raw propolis on ruminal fermentation using RUSITEC and found that dietary treatment with propolis had no significant (P>0.05) effect on gross energy intake as compared to control ( devoid of propolis or moringa by-products). However, in an *in vitro* study by Coşkuntuna *et al*. (2023), who studied the effects of adding 0.05% propolis extract to sorghum grain varieties (Es8z102, Albanus, Sugar Drip, Gül Şeker and Csr9303) containing different levels of tannins, reported that ME availability increased significantly (P=0.000) in response to addition of propolis extract to all the sorghum grain varieties as compared to the control (without addition of propolis extract).

However, the rumen ammonia nitrogen ($NH_3$-N, mg/dl) showed a significant decline (P≤0.05) at 0.2% inclusion level of propolis (17.79 mg/dl) as compared to the other propolis levels (Table 4). The results of the current study resembled with the results of Ehtesham *et al*. (2018) who used an *in vitro* gas production system to investigate the effects of varying concentrations of phenolic compounds found in Iranian propolis (IP) extracts (25, 50, and 75 g of propolis in 100 ml of 70% ethanol) on rumen fermentation using two rations, high concentrate ration (HC) and middle concentrate ration (MC) with concentrate to forage ratios as 80:20 and 60:40, respectively. They reported that in MC ration, addition of 75% of IP significantly decreased (P<0.0001) $NH_3$-N levels as compared to control as well as other propolis levels (25 and 50 g of propolis). In HC ration, addition of 50% and 75% of IP significantly decreased (P<0.0001) $NH_3$-N levels as compared to control and propolis treatment containing 25% of IP. Similarly, Özturk *et al*. (2010) studied the effects of varying concentrations of propolis extract (0%, 20%, 60% ethanolic extract of propolis) using RUSITEC and revealed that propolis extract has the ability to lower (*P*<0.05) rumen $NH_3$-N levels in a dose-dependent manner, reducing concentrations by 24% and 39% at low (20%) and high (60%) concentrations of propolis, respectively as compared to control (0% propolis extract). Lower rumen $NH_3$-N levels in ruminants can offer several advantages, including reduced nitrogen losses, improved feed efficiency, and potentially better animal health. Lower $NH_3$-N levels also contribute to decreased ammonia emissions from manure.

## CONCLUSION

The net gas production, digestibility of nutrients (OM, NDF, DM) and availability of metabolizable energy were similar among various levels of bee propolis tested in maize silage based TMR, indicating that levels of bee propolis had no adverse effect on nutrient digestibility. However, ammonia production reduced significantly (P≤0.05) in the *in vitro* medium without affecting feed digestibility and microbial mass production at @ 0.2 percent (DM basis) level of bee propolis. The data conclusively revealed that bee propolis could be added at 0.2% (DM basis) in the maize silage based TMR for ruminants with a potential to decrease nitrogen losses.

## REFERENCES


Aguiar, S.C.D., Paula, E.M.D., Yoshimura, E.H., Santos, W.B.R.D., Machado, E., Valero, M.V., Santos, G.T.D. and Zeoula, L.M. 2014. Effects of phenolic compounds in propolis on digestive and ruminal parameters in dairy cows. *Rev. Bras. Zootec.*, **43**: 197-206.

AOAC. 2007. Official Methods of Analysis, 18th edition. Association of Official Analytical Chemists, Arlington, Virginia, USA.

Blümmel, M., Makkar, H.P.S. and Becker, K. 1997. *In vitro* gas production: a technique revisited. *J. Anim. Physiol. Anim. Nutr. (Berl).*, **77**(1-5): 24-34.

Coşkuntuna, L., Erten, K. and Şamli, H.E. 2023. Effect of adding propolis extract to sorghum grain varieties on *in vitro* gas parameters. *J. Elem.*, **28**(2): 485-496.

Da Silva, N.I.S., De Araújo, T.L.C., Firmino, S.S., Oliveira, M.R., De Sousa, B.P., Da Costa, R.M.N., Macedo, M.F., Mendes, A.C.D., Pereira, M.W.F., De Almeida, J.G.L., De Oliveira Lima, P. and De Lima Júnior, D.M. 2025. Green propolis extract derived from *Mimosa tenuiflora* as an additive in the diet of sheep. *Trop. Anim. Health Prod.* , **57**(2): 57-67.

DAHD. 2024. Brochure of the 21st Livestock Census. Ministry of Fisheries, Animal Husbandry and Dairying.

De Paula, E.M., Samensari, R.B., Machado, E., Pereira, L.M., Maia, F.J., Yoshimura, E.H., Franzolin, R., Faciola, A.P. and Zeoula, L.M. 2016. Effects of phenolic compounds on ruminal protozoa population, ruminal fermentation and digestion in water buffaloes. *Livest. Sci.*, **185**: 136-141.

Ehtesham, S., Vakili, A.R., Danesh Mesgaran, M. and Bankova, V. 2018. The effects of phenolic compounds in Iranian propolis extracts on *in vitro* rumen fermentation, methane production and microbial population. *Iran. J. Appl. Anim. Sci.*, **8**(1): 33-41.

Fallah, M., Najafi, F. and Kavoosi, G. 2021. Bee propolis, bee bread and royal jelly: Proximate analysis, fatty acid composition, nutritional quality and anti-amylase activity. *Res. Sq.*: 1-26. https://doi.org/10.21203/rs.3.rs-927023/v1

France, J., Dhanoa, M.S., Theodorou, M.K., Lister, S.J., Davies, D.R. and Isac, D. 1993. A model to interpret gas accumulation profiles associated with *in vitro* degradation of ruminant feeds. *J. Theor. Biol.*, **163**(1): 99-111.

Heimbach, N.S., Ítavo, C.C.B.F., Ítavo, L.C.V., Franco, G.L., Leal, C.R.B., Leal, E.S., Silva, P.C.G., Rezende, L.C. and Silva, J.A. 2014. Residue from extraction of brown propolis in the diet to ruminants: *in vitro* digestibility and gas production. *Arch. Zootec.,* **63**(242): 259-267.

Madras-Majewska, B., Ochnio, L. and Ochnio, M. 2015. Use of bee products in livestock nutrition and therapy. *Med. Weter.*, **71**(2): 94–99.

Mahmood, M., Samli, H.E., Sener-Aydemir, A., Sharma, S., Zebeli, Q. and Khiaosa-ard, R. 2022. Moringa oleifera and Propolis in Cattle Nutrition: Characterization of Metabolic Activities in the Rumen In Vitro. *Metabolites*, **12**(12), 1237.

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W. 1979. The estimation of the digestibility and metabolizable energy content of ruminant feeding stuffs from the gas production when they are incubated with rumen liquor *in vitro*. *J. Agric. Sci*., **93**(1): 217–222.

Menke, K.H. and Steingass, H. 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. *Res. Dev*., **28**: 7-55.

Morsy, A.S., Soltan, Y.A., Elzaiat, H.M., Sallam, S.M.A., Alencar, S.D., Louvandini, H. and Abdalla, A.L. 2011. Effect of two types of Brazilian propolis extracts on rumen gas and methane production and truly degradability *in vitro*. *In:* Proceedings of the 4th Animal Wealth Research Conference in the Middle East & North Africa. Oct, 2011. Egypt. pp. 01–20.

Morsy, A.S., Soltan, Y.A., Sallam, S.M.A., Kreuzer, M., Alencar, S.M.D. and Abdalla, A.L. 2015. Comparison of the *in vitro* efficiency of supplementary bee propolis extracts of different origin in enhancing the ruminal degradability of organic matter and mitigating the formation of methane. *Anim. Feed Sci. Technol*., **199**: 51-60.

Morsy, A.S., Soltan, Y.A., El-Zaiat, H.M., Alencar, S.M.D. and Abdalla, A.L. 2021. Bee propolis extract as a phytogenic feed additive to enhance diet digestibility, rumen microbial biosynthesis, mitigating methane formation and health status of late pregnant ewes. *Anim. Feed Sci. Technol.*, **273**: 114834.

Nascimento, R.J.T., Teixeira, R.M.A., Tomich, T.R., Pereira, L.G.R., Carmo, T.D.D., Neiva, A.P., Junior and Cappelle, E.R. 2020. Residue of propolis extract in bovine diets with increasing levels of protein on rumen fermentation. *Pesqui. Agropecu. Bras.*, **55**: (e01572).

Özturk, H., Pekcan, M., Sireli, M. and Fidanci, U.R. 2010. Effects of propolis on *in vitro* rumen microbial fermentation. *Ank. Univ. Vet. Fak. Derg.*, **57**(4): 217-221.

Pant, K., Thakur, M., Chopra, H.K., Nanda, V., Ansari, M.J., Pietramellara, G., Pathan, S.I., Alharbi, S.A., Almoallim, H.S. and Datta, R. 2021. Characterization and discrimination of Indian propolis based on physicochemical, techno-functional, thermal and textural properties: A multivariate approach. *J. King Saud Univ. Sci.,* **33**(1): 101405.

Sierra-Galicia, M.I., Rodríguez-de Lara, R., Orzuna-Orzuna, J.F., Lara-Bueno, A., García-Muñiz, J.G., Fallas-López, M. and Hernández-García, P.A. 2022. Supplying bee pollen and propolis to growing rabbits: Effects on growth performance, blood metabolites and meat quality. *Life*, **12**(12): 1987.

SPSS. 2012. Statistical package for windows. Chicago, IL, USA.

Valero, M.V., Zeoula, L.M., Moura, L.P.P., Júnior, J.B.G.C., Sestari, B.B. and Prado, I.N.D. (2015). Propolis extract in the diet of crossbred (½ Angus vs. ½ Nellore) bulls finished in feedlot: animal performance, feed efficiency and carcass characteristics. *Semina. Cienc. Agrar.,* **36**(2): 1067-1078.

Van Soest, P.V., Robertson, J.B. and Lewis, B. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci*., **74**(10): 3583-3597.

Wagh, V.D. 2013. Propolis: a wonder bees product and its pharmacological potentials. *Adv. Pharmacol. Pharm. Sci.*, **2013**: 1–11.