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Short Communication

EFFECT OF GRADED LEVELS OF BEE PROPOLIS ON FERMENTATION CHARACTERISTICS OF MAIZE FODDER BASED RATION: AN *IN VITRO* ASSAY

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SUMMARY

An *in vitro* assay was carried out to evaluate the fermentation parameters of supplementing graded levels of bee propolis to maize fodder based ration. Maize fodder based total mixed ration (TMR) was supplemented with bee propolis at 0, 0.025, 0.05, 0.1, 0.15 and 0.2% (on DM basis). The addition of graded levels of bee propolis had no significant effect on the fermentation parameters, such as net gas production, partitioning factor, digestibility of nutrients (OM, NDF, DM), microbial mass production, efficiency of microbial mass production, production of short chain fatty acids, and availability of metabolizable energy. However, at 0.2% (DM basis) inclusion level of bee propolis, ammonia-N (NH_3-N) production decreased significantly ($P \geq 0.05$) in the *in vitro* media. It was concluded that adding bee propolis at 0.2% level (DM basis) in the maize fodder based diet effectively reduced NH_3-N production, thereby, indicating its potential to decrease ruminal NH_3-N nitrogen during fermentation in animals.

Keywords: Ammonia-N, Bee propolis, *In vitro* evaluation, Maize fodder based ration

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The growing global human population puts enormous pressure on livestock farmers to increase productivity, maintain animal health, and improve feed to meet the growing demand for meat, dairy, and eggs; this pressure is further accentuated by concerns about environmental sustainability and animal welfare. These objectives have long been met by the use of synthetic feed additives and antibiotics; however, rising awareness about antimicrobial resistance and persistence of chemical residues in livestock based products and consumer preference for natural and organic food sources have fuelled the search for safer, sustainable and organic alternatives for animal feed supplementation. In this context, bee propolis has gained significant attention of scientists as a potential feed additive, owing to its rich chemical composition and biological properties (Abd El Ghany, 2024).

Propolis is composed of resins and bees wax gathered by the honey bees from various plants and its parts like flowers and leaf buds; and plays protective role by sealing the cracks and crevices in the hive (Bogdanov, 2017). As propolis naturally contains potent bioactive compounds, especially flavonoids, phenolic acids, steroids, alcohols, fatty acids and ketones, it has antimicrobial, antimethanogenic, anti-inflammatory, immunomodulatory, anticarcinogenic and antiparasitic properties (Morsy *et al.*, 2016). Considering the broad spectrum of useful properties of bee propolis, an *in vitro* study was conducted to investigate its impact on various fermentation parameters.

An *in vitro* assay was carried out at Department of Animal Nutrition, GADVASU (Ludhiana, Punjab). All the experimental protocols and procedures described here were approved by the guidelines of the Institutional Animal Ethics Committee. The bee propolis was sourced from local market.The roughage to concentrate ratio in the TMR was 65:35, with the roughage part consisting of wheat straw and maize fodder in 50:50 ratio. Graded levels of propolis at 0, 0.025, 0.05, 0.1, 0.15, and 0.2% (DM basis) were added to maize fodder-based TMR. Bee propolis and maize fodder-based TMR were analyzed for proximate (AOAC, 2005) and cell wall components (Van Soest *et al.*, 1991).

Ruminal fluid was collected from male buffaloes having rumen fistula, that were maintained on ration comprising of 2 kg conventional concentrate mixture, 15 kg fresh green forage and 3 kg wheat straw. *In vitro* evaluation was carried out by gas production technique (Menke *et al.*, 1979; Menke and Steingass, 1988). The ratio of substrate actually degraded *in vitro* (mg) to the amount of gas produced (ml) by it was used to calculate the partitioning factor (PF) (France *et al.*, 1993). The ME value of the substrate was determined using the equation given by Menke *et al.* (1979). Simple ANOVA was used to analyze the data generated throughout the investigation using SPSS version 21. Tukey's b was used to test the mean differences.

The bee propolis used in the study contained 95.63% organic matter, 4.37% total ash, 2.51% crude protein, 1.10% ether extract, 0.60% crude fibre and 92.02% total carbohydrates. The ingredient composition of TMR is

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given in Table 1. In the TMR, the roughage portion was made up of wheat straw and maize fodder in 1:1 ratio. TMR contained 12.40% crude protein and 2.85% ether extract (Table 2).

Table 1. Ingredient composition of TMR used in the *in vitro* experiment (% DM basis)

Ingredient	Percent
Wheat straw	32.5
Maize fodder	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 2. Chemical composition of maize fodder based TMR used in the *in vitro* experiment (% DM basis)

Parameter	Percent
Organic matter	93.12
Total ash	6.88
Crude protein	12.40
Ether extract	2.85
Neutral detergent fibre	56.90
Acid detergent fibre	31.15
Hemicellulose	25.75
Cellulose	21.70
Acid detergent lignin	4.35
Total carbohydrates	77.87

The *in vitro* fermentation parameters of maize fodder based TMR are presented in Table 3. There was no significant difference in net gas production (NGP) among the various levels of bee propolis tested in the TMR. Our results are similar to those reported by Nascimento *et al.* (2020), who stated that addition of propolis extraction residue (100 g/cow/day) into bovine diets (with varying concentrate levels viz. 25, 50 and 75% DM) *in vitro* had no significant effect on total volume of gases produced in the treatments as compared to control group (without propolis extraction residue). Moreover, Morsy *et al.* (2015) compared the effects of increasing levels of ethanolic extracts of Brazilian red propolis and Egyptian brown propolis on ruminal degradation of nutrients and methane productionusing a semi-automatic *in vitro* gas production system and found no variation in NGP at various propolis levels. Further, Morsy *et al.* (2011) found no significant ($P>0.05$) difference in gas production between Brazilian green propolis extract (BGP) and Brazilian alamopropolis extract (BAP) at various levels (125, 250 and 500 µg/75 ml culture fluid) in comparison to control.

The partitioning factor (PF) in the current study varied non-significantly across treatments. Our results are in line with those of Morsy *et al.* (2015), who compared the effects of increasing levels of ethanolic extracts of Brazilian red propolis and Egyptian brown propolis on ruminal degradation of nutrients and methane production using a semi-automatic *in vitro* gas production system, and reported no significant difference in the PF values across the propolis treatments. In addition, Morsy *et al.* (2011) observed no significant difference in the PF values among both Brazilian propolis extracts-BGP and BAP at different levels (125, 250 and 500 µg/75 ml culture fluid) in comparison to control. The PF value of ruminant ration should lie in the range of 2.71 to 4.41 (Blümmel *et al.*, 1997). The PF value (mg/ml) in the present study ranged from 3.33 to 3.57, which is within the suggested range.

The digestibility of nutrients (OM, NDF and DM) varied non significantly across various levels of bee propolis supplementation in the TMR. Our results are consistent with those of Mahmood *et al.* (2022), who used RUSITEC to study the effects of different moringa by-products and raw propolis on ruminal fermentation, and reported that there wasno significant ($P>0.05$) variation in the degradation of nutrients across the treatments (control diet without any feed supplement and the control diet added with moringa byproducts or raw propolis). Further, Nascimento *et al.* (2020) investigated the effects of adding propolis extraction residue (100 g/cow/day) into bovine ration with varying levels ofconcentrate on in vitro rumen fermentation, and reported that addition of propolis extraction residue into bovine ration had no significant effect on digestibilityof DM at variouslevels of concentrate as compared to control (without propolis extraction residue).

The supplementation of graded levels of bee propolis in to TMR did not have any significant effect on microbial mass production (MMP) and efficiency of microbial mass production (EMMP). Our results are in line with that of Valero *et al.* (2015), who studied the substitution of propolis for monensin in the diet of feedlots bulls, and reported that both MMP and EMMP were unaffected ($P>0.05$) by the addition of monensin or propolis. Further, Aguiar *et al.* (2014) investigated the effect of inclusion of three variations of propolis based products (PBP) as PBP B1, PBP C1 and PBP C3 (varying in the concentrations of phenolic compounds as 3.81 mg/kg, 3.27 mg/kg and 1.93 mg/kg of ingested dry matter,

Table 3. Effect of level of bee propolis on the *in vitro* gas production and digestibility of nutrients in maize fodder 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)	169.33	168.67	167.33	172.67	175.33	174.67	1.03
PF (mg/ml)	3.57	3.39	3.45	3.33	3.48	3.33	0.03
OMD (%)	64.92	61.34	62.05	61.77	65.63	62.48	0.62
NDFD (%)	42.59	36.73	37.90	37.43	43.76	38.61	1.01
MMP (mg)	86.99	75.04	78.64	73.24	84.54	74.09	1.99
EMMP (%)	38.35	35.03	36.29	33.94	36.82	33.96	0.58
DMD (%)	68.27	65.47	66.00	66.13	69.60	67.07	0.54
SCFA (mmole)	0.75	0.74	0.74	0.76	0.77	0.77	0.00
ME (MJ/kg DM)	7.68	7.66	7.62	7.78	7.86	7.84	0.03
NH3-N (mg/dl)	20.39^{ab}	21.55^{b}	20.97^{ab}	20.68^{ab}	20.82^{ab}	19.37^{a}	0.22

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, NH3-N- Ammoniacal nitrogen, R:C ratio was 65:35 on dry matter basis
Means bearing different superscripts in a row differ significantly ($P \geq 0.05$)

respectively) to the diets of Holstein cattle and reported that there was no significant (P>0.05) variation in the microbial protein synthesis as well as microbial efficiency on addition of propolis-based products in the cattle ration as compared to control. Moreover, De Paula *et al.* (2016) studied the effects of offering propolis-derived phenolic compounds to water buffaloes at various doses (0, 16.95, 33.9 and 50.85 mg/d), and revealed that there were no significant variations (P>0.05) in MMP as well as EMMP among control and treatment groups.

Adding graded levels of bee propolis to TMR had no significant effect on the production of short chain fatty acids (SCFA).Our results are similar to those of Mahmood *et al.* (2022), who used RUSITEC to study the effects of various moringa by-products and raw propolis on ruminal fermentation and reported that propolis treatment had no significant (P>0.05) effect on the concentration of total SCFA as compared to control (devoid of feed additive). In the present study, adding graded levels of bee propolis to TMR had no significant effect on ME availability. Our results resembled with those of Da Silva *et al.* (2025) who explored the effects of supplementation of increasing levels (0, 6, 12, 18 and 24 ml/day) of green propolis extract (GPE) in ration of feed lot rams for 85 days and reported that ME in the rams' diet was not affected (P > 0.05) by the supplementation of GPE across treatment groups. However, Coşkuntuna *et al.* (2023) carried out an *in vitro* study to investigate effects of adding 0.05% propolis extract to sorghum cultivars (Es8z102, Albanus, Sugar Drip, GülŞeker and Csr9303) having varying levels of tannins and reported that availability of ME increased significantly (P = 0.000) with the addition of propolis extract to all the sorghum cultivars in comparison to control (without propolis extract).

However, *in vitro* NH_3-N (mg/dl) varied significantly ($P \geq 0.05$) across the various levels of propolis with the highest ($P \geq 0.05$) value (21.55 mg/dl) recorded at 0.025% level of propolis and lowest ($P \geq 0.05$) value (19.37 mg/dl) recorded at 0.2% level of propolis. The results of the our study corroborated with those of Ehtesham *et al.* (2018), who used an *in vitro* gas production system to explore the effects of increasing levels 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. They revealed that addition of 75% IP in ration significantly reduced (P<0.0001) levels of NH_3-N in comparison to control as well as other propolis levels (25 and 50 g of propolis). Lower rumen NH_3-N levels in ruminants can offer several advantages, including improved nitrogen utilization by rumen microbes and reduced nitrogen waste excretion. This enhances feed efficiency, improves animal health and lowers environmental pollution. Lower rumen NH_3-N levels decrease energy expenditure on excretion of ammonia as urea, thereby, improving energy efficiency and animal performance (Chanu *et al.*, 2020).

CONCLUSION

Net gas production, nutrient digestibility (OM, NDF and DM), microbial mass production,and availability of ME were similar across different bee propolis levels used in maize fodder-based TMR, suggesting that bee propolis levels had no negative impact on nutrient digestibility. However, at a 0.2 % (DM basis) inclusion level of bee propolis, NH_3-N production decreased significantly ($P \geq 0.05$) in the *in vitro* medium.

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