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Evaluating the methane mitigation potential of ginger rhizomes *(Zingiber officinale)* by *in vitro* study

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Abstract

The study explores the use of ginger rhizomes (Zingiber officinale) to mitigate methane emissions in livestock. In vitro evaluations were conducted to analyse the nutritional composition, phytochemical profile and methane mitigation potential of ginger rhizomes. The crude protein, crude fibre, ether extract, total ash and nitrogen free extract of ginger rhizomes (Zingiber officinale) were evaluated. The phytochemical screening of ginger rhizomes (Zingiber officinale) was qualitatively assessed in water, methanol and ethanol extracts. Further the different dose levels of ginger rhizomes (Zingiber officinale) at 20, 40, 60, 80 and 100 mg were evaluated in vitro for its methane mitigation potential. The dry matter, crude protein, crude fibre, ether extract, total ash and nitrogen free extract of ginger rhizomes (Zingiber officinale) were 20.58, 0.78, 11.89, 6.30, 5.45 and 75.58 per cent respectively. The phytochemical screening of alkaloids, flavonoids, tannins, phenol, saponins, carbohydrates, proteins, amino acids, phytosterols, terpenoids indicated that they are present in the water, methanol and ethanol extracts. The ginger rhizomes (Zingiber officinale) at dose level of 80 mg and 100 mg produce 6.74 and 5.79 ml of methane per 100 mg of truly digested substrate by in vitro study. These dose levels have significantly ($p<0.05$) higher methane mitigation potential than the other dose levels.

Keywords: *Methane, in vitro, digestibility, ginger rhizomes*

The ruminant's digestive system has different types of microorganisms responsible for conversion of feed into finished products leading to production of various volatile fatty acids such as acetic, propionic and butyric acids, carbon dioxide and methane and small quantities of iso butyric acid, valeric acid, 2- methyl butyric acid, 3-methyl butyric acid. Methane is second major gas after carbon dioxide responsible for environmental warming and ozone layer depletion. It is potent greenhouse gas and it has 23 times higher global warming potential than carbon dioxide (IPCC, 1996). Herbal products are of organic nature and don't produce harmful deleterious residual effects in the animal products and they are used by human beings for centuries without adverse effects. A commonly used spice and condiment is *Zingiber officinale* Roscoe (Zingiberaceae), commonly known as ginger. The active compounds are non -volatile pungent principles, namely gingerols, shogaols, paradols, and zingerones. The phytochemicals present in the ginger rhizomes are alkaloids (11.21%), tannins (3.54%), carotenoids (0.64 µg/100 gram), saponin (0.80%), flavonoids (5.56%), steroids (0.04%), cardenolides (0.02%) as reported by Gloria *et al.* (2010). Kim *et al.* (2012) reported that worm wood

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(*Artemisia absinthium*), garlic (*Alium sativum*), onion (*Alium cepa*), ginger (*Zinginber officinalis*), mandarin orange (*Citrus reticulata*), and honeysuckle (*Lonicera sempervirens*) plant extracts were used and they all reduced the population of ciliate-associated methanogen, thereby, minimising the methane emissions. Chao and Young (2000) observed that roots of *Zingiber officinale* (ginger) are rich in camphene (14.1%), β-bisabolene (22.1%) and ar-curcumene (14.5%) which has the anti-methanogenic activity. Herbal feed additives like *Zingiber officinale* (ginger) offer a promising, eco-friendly solution due to their bioactive compounds (gingerols, shogaols, paradols, zingerones) and phytochemicals (alkaloids, flavonoids, tannins, etc.), which demonstrate anti-methanogenic properties. Using such natural alternatives is not only safe for animals and humans but also avoids harmful residues in animal products, aligning with the global push for sustainable livestock production. Although previous studies (Kim *et al.,* 2012; Chao and Young, 2000) reported that ginger and other herbal extracts reduce ciliate-associated methanogens, the exact mechanisms and efficiency of ginger rhizomes in mitigating methane production remain insufficiently explored, especially in *in vitro* ruminant fermentation models. Furthermore, the specific role of ginger's key bioactive compounds (camphene, β-bisabolene, ar-curcumene) in methane reduction needs further validation.

This study aims to fill that gap by specifically evaluating the methane mitigation potential of ginger rhizomes under controlled conditions. The present study is designed to evaluate methane mitigation potential of the ginger rhizomes in *in vitro* study. To analyse the phytochemical composition of ginger rhizomes, focusing on bioactive compounds that may influence microbial fermentation and methane production. To evaluate the effect of ginger rhizomes on methane production during *in vitro* ruminant digestion.

Materials and methods

The proximate principles of crude protein, ether extract, crude fibre, nitrogen free extract, and total ash of ginger rhizomes were analysed as per AOAC (2019). The plant extracts were prepared in three solvents *viz*., water, ethanol (95/100 ml) and methanol (98/100 ml) at 20 g per 100 ml of solvent for phytochemical evaluation as per the method (Patra *et al.* 2006). The presence of alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids, phytosterols, terpenoids in aqueous, methanol and ethanolic extract were evaluated as per procedure (Tiwari *et al.* 2011). As per the procedure of Menke and Steingass (1988), the *in vitro* gas production studies were carried out using Hohenheim gas production technique in substrates. The rumen liquor collected from the cattle fed with Total Mixed Ration (TMR). The methane concentration at different dosage of ginger rhizomes at 20, 40, 60, 80, 100 mg and in control group was estimated as per procedure of Sitaula *et al.* (1992) using gas chromatography (Perkin Elmer, Clarus 500 model) equipped with Flame Ionization Detector (FID) and capillary column (30-meter length and 250 micrometer diameter).

The *in vitro* true dry matter digestibility of the fermented feed was estimated as per Van Soest and Robertson, (1988). The true dry matter digestibility was calculated as the weight of the sample incubated minus the weight of the residue after Neutral Detergent Soluble (NDS) treatment. Methane (ml) for 100 mg of truly digested substrate was calculated based on methane production and *in vitro* dry matter digestibility of substrate incubated using following formula

$$\text{Methane production per 100 mg truly digested substrate} = \frac{\text{Methane emission (ml)}}{\text{Degradability (\%)}} \text{ X } 0.1 \text{ gm}$$

Statistical analysis

Data obtained from *in vitro* studies were analysed with analysis of variance (ANOVA) using IBM, SPSS statistics version 20.0 for windows software as per the Snedecor and Cochran (1994). The critical difference between the groups was analysed as per Duncan's multiple range test.

Results and discussion

Chemical composition of ginger rhizomes (Table 1) contained 0.78, 11.89,6.30, 5.45 and 75.58 per cent of crude protein, crude fibre, ether extract, total ash and nitrogen free extract respectively on dry matter basis. The proximate values obtained in this study revealed a higher CP, CF, EE and TA content than that reported by Osabor *et al.* (2015) in ginger rhizomes. In contrast to the findings in the present study, Gloria *et al.* (2010); Shrin and Prakash, (2010) reported higher CP content of 8.58, 11.65, 9.54, 7.68, 5.08%, respectively in ginger rhizomes. Ajayi *et al.* (2013) also reported higher CF and EE content of 21.90 and 17.11%, respectively compared to the findings in the present study. In contrary to the present findings, Medjekal (2017) recorded very high total ash content of 17.80% in ginger rhizomes.

Table 1. Chemical composition of ginger rhizomes (%DM Basis)

Parameters	Content (%)
Dry matter	20.58± 0.36
Crude protein	8.78 ± 0.02
Crude fibre	11.89 ± 0.25
Ether extract	6.30 ± 0.18
Total ash	5.45 ± 0.32
NFE	67.58 ± 0.56

Each value is mean of six observations

Table 2. Phytochemical screening of ginger rhizomes

Phytochemicals	Tests	Water	Methanol	Ethanol
Alkaloids	Wagner's test	+	-	+
Flavonoids	Lead acetate test	+	+	+
Tannins	Gelatin test	+	+	-
Phenols	Ferric chloride test	+	+	-
Saponins	Foam test	+	+	-
Carbohydrates	Benedict's test	+	+	+
Proteins	Xanthoproteic test	+	+	+
Aminoacids	Ninhydrin test	+	+	-
Phytosterols	Salkowski's test	+	+	-
Terpenoids	Salkowski's test	+	+	+

Each value is mean of six observations

Table 3. *In vitro* study of ginger rhizomes at different levels

Parameters	Control	20 mg	40 mg	60 mg	80 mg	100 mg
Total gas (mL)	$58.33^{d} \pm 2.50$	$48.17^{c} \pm 2.87$	$45.83^{b} \pm 2.33$	$45.50^{b} \pm 8.84$	$42.74^{ab} \pm 6.014$	$39.33^{a} \pm 8.66$
Methane production (mL)	$12.54^{d} \pm 1.38$	$9.66^{c} \pm 1.86$	$9.0^{b} \pm 2.09$	$8.16^{b} \pm 4.08$	$7.85^{ab} \pm 1.47$	$6.66^{a} \pm 1.03$
Percentage of methane on total gas production	$21.91^{d} \pm 2.43$	$20.07^{c} \pm 3.18$	$19.63^{bc} \pm 2.71$	$17.95^{ab} \pm 2.50$	$18.37^{ab} \pm 1.75$	$16.94^{a} \pm 2.13$
True dry matter digestibility (%)	$65.39^{e} \pm 5.64$	$54.35^{ab} \pm 5.91$	$60.20^{cd} \pm 8.07$	$57.45^{bc} \pm 5.54$	$61.85^{d} \pm 5.91$	$53.05^{a} \pm 6.64$
Methane production per 100 mg of truly digested substrate (mL)	$10.98^{c} \pm 1.83$	$8.33^{b} \pm 1.85$	$7.98^{b} \pm 1.47$	$7.68^{b} \pm 1.13$	$6.74^{ab} \pm 1.48$	$5.79^{a} \pm 1.16$
p value	0.024	0.051	0.023	0.015	0.035	0.027

*Each value is the mean of six observations

Means bearing different superscripts in a row differ significantly ($P<0.05$).

The alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids and phyto sterols evaluated in aqueous, methanol and ethanol extracts are presented in the Table 2. The phyto chemical screening of ginger rhizomes indicated that alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids and phyto sterols present in aqueous and methanol extracts. The data revealed that ginger rhizomes contained alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids and phyto sterols in water extract. However, alkaloids were absent in methanol extract. Further, the study also revealed that in ethanol extract, there is absence of tannins, phenols, saponins, amino acids and phyto sterols. Khanpara *et al.* (2012) also reported similar presence of saponin, alkaloids, sugar, flavonoids and contrarily, except tannin in shikakai. Osabor *et al*. (2015) also observed the presence of alkaloids, flavonoids, saponins, poly phenols in aqueous extract and reported absence of tannins. Contrary to the present findings, Setty *et al.* (2011). observed presence of all phytochemicals viz., alkaloids, flavanoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids and phytosterols in ethanolic extract in comparison to absence of tannins, phenols, saponins, amino acids and phytosterols in ethanolic extract in the present study.

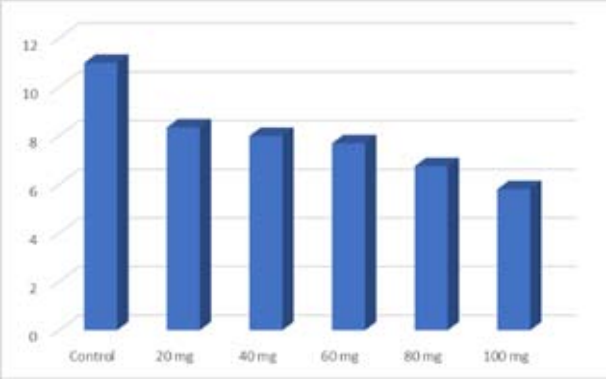

Fig 1. Effect of different level of ginger rhizomes on methane mitigation (methane production per 100 mg truly digested substrate in mL)

Total gas production, methane production, percentage of methane on total gas production, true dry matter digestibility and methane production per 100 mg truly digested substrate evaluated are presented in Table

3. The total gas production estimated *in vitro* in control, 20 mg, 40 mg, 60 mg, 80 mg, and 100 mg are 58.33, 48.17, 45.83, 45.50, 42.74 and 39.33 mL, respectively. The results of methane production of ginger rhizomes studied at control, 20 mg, 40mg, 60 mg, 80 mg and 100 mg dose levels are 12.54, 9.66, 9.0, 8.16, 7.85 and 6.66 mL respectively. Significantly (P<0.05) lower methane gas production was observed in 80 mg and 100mg dose level to other treatment and control groups. Similarly, Sirohi *et al.* (2009) observed acetone extracts of shikakai pods (*Acacia concinna*) significantly lowered the methane production (28.15 ml per gram DM) over control group (37.55 ml per gram DM). Patra *et al.,* (2006) reported that water extract of ginger rhizomes had no effect on methane production.

The true dry matter digestibility of ginger rhizomes at control, 20 mg, 40 mg, 60 mg, 80 mg and 100 mg dose level was 65.39, 54.35, 60.20, 57.45, 61.85 and 53.05 per cent, respectively. Significantly higher true dry matter digestibility (p<0.05) was observed in control and in treatment group of ginger rhizomes containing dose levels of 80 mg. Similar to the findings in the present study, Patra *et al.* (2006) observed that addition of shikakai pods extracts (water, ethanol, methanol) to the wheat straw and concentrate based rations resulted *in vitro* Organic Matter Digestibility (IVOMD) was reduced (P<0.05) over the control group. The methane production per 100 mg of truly digested substrate in control, 20 mg, 40mg, 60 mg, 80 mg and 100 mg dose levels are 10.98, 8.33, 7.98, 7.68, 6.74 and 5.79 ml, respectively. The methane production per 100 mg of truly digested substrate is significantly (P<0.05) lower in 100 mg dose level compared to control and other dose levels of ginger rhizomes studied. Higher dose level of ginger rhizomes decreases the methane production and methane production per 100 mg substrate. Kim *et al.* (2012) observed similar results of this present study lower methane level in ginger extract (*Zingiber officinale*) than in control group.

Conclusion

It could be concluded that among the different doses studied *in vitro* ginger rhizomes, 80 mg and 100 mg dose levels lowered methane production per 100 mg truly digested substrate compared to the control and other dose levels.

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Conflict of interest

The authors have no conflicts of interest to declare.

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