

## Evaluation of Chemical, Phyto Chemical and Methane Mitigation Potential of Turmeric (*Curcuma longa*) by *In-vitro* Study

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### Abstract

*The nutritional composition, phytochemical and methane mitigation potential of turmeric (*Curcuma longa*) was evaluated. Turmeric contained 14.34, 14.73, 5.42, 2.34, 9.52 and 53.65 per cent moisture, crude protein, crude fibre, ether extract, total ash and nitrogen free extract, respectively. Qualitative assessment of Phytochemical screening of alkaloids, flavonoids, tannins, phenol, saponins, carbohydrates, proteins, amino acids, phytosterols and terpenoids in water and methanol extract revealed presence of all these compounds except proteins and amino acids. The turmeric was also evaluated in 20mg, 40mg, 60mg, 80 mg, and 100mg dose level in in vitro rumen fermentation studies for its methane mitigation potential. The highest methane mitigation potential (4.34±1.14) i.e. methane production per 100 mg truly digested substrate was observed in 80 mg turmeric dose level which was significant (P<0.05). It could be concluded that turmeric is a good protein source and its supplementation at 80 mg dose level in vitro reduced methane production.*

**Keywords:** Chemical composition, In- vitro and Methane, Phyto Chemical, Turmeric



## Introduction

Turmeric (*Curcuma longa*) is one of the important medicinal plant used for Ayurvedic, Siddha and Unani medicines in India. The rhizomes are traditionally known as “Haldi”. Dried powder gives yellow colour. Turmeric is also used in traditional medicine to cure gastro intestinal diseases particularly biliary and hepatic disorder, diabetic wounds, rheumatism, inflammation, sinusitis, anorexia, coryza and cough. Further, turmeric is supposed to contain properties like anti-cancer, anti-diabetic, anti-oxidant, hypolipidemic, anti-inflammatory, anti-microbial, anti-fertility, anti-venom, hepato protective, nephron protective, anti-coagulant and anti-HIV activity. Phenolic di ketone, curcumin (diferuloylmethane) (3-4%) is responsible for yellow colour and constitutes curcumin I (94%), curcumin II and curcumin III (6%) (Chanda and Ramachandra, 2019). The present study was designed to evaluate the chemical, phyto chemical and methane mitigation potential of turmeric *In-vitro*.

## Materials and Methods

The proximate principles in turmeric viz., moisture, crude protein, ether extract, crude fibre, nitrogen free extract, and total ash were estimated as per AOAC (2016). For phytochemical evaluation, the plant extracts were prepared in four solvents viz., water, ethanol (95/100 ml), methanol (98/100 ml) and chloroform (100 ml) at 20 g per 100 ml of solvent. For the preparation of water extract, plant materials were boiled for 5 min on a low flame. The flasks of all the solvents were stoppered and incubated at 39°C on a rotary shaker for 24 h and filtered through Whatman filter paper no. 1. The filtrates were collected and stored at 4°C for further use (Patra *et al.*, 2006). The preliminary phytochemicals evaluation of aqueous extract, methanolic extract, ethanolic extract, and chloroform extract of turmeric was performed for the presence of alkaloids flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids, phytosterols, terpenoids (Tiwari *et al.*, 2011). The *in vitro* gas production studies were carried out using Hohenheim gas production technique as per the procedure of Menke and Steingass (1988). Methane concentration in the gas produced during fermentation 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). Nitrogen gas was used as carrier gas with temperatures of oven, injector and detector maintained at 60, 100 and 110°C respectively. The collected gas sample in vacutainer was injected in to injector column and detected in detector. Before running of sample, the equipment was conditioned and run with standard sample of known concentration. Methane (%) concentration in samples and methane emission was calculated using the following formulae-

$$\text{Methane concentration(\%)} = \frac{\text{Peak area of sample gas}}{\text{Peak area of standard gas}} \times \text{Methane concentration in standard gas}$$

$$\text{Methane emission(ml)} = \frac{\text{Methane concentration(\%)}}{100} \times \text{Net gas production(ml)}$$

Percentage of methane in total gas produced was calculated using the following formula-

$$\text{Methane percentage on total gas production} = \frac{\text{Methane emission(ml)}}{\text{Total gas(ml)}} \times 100$$

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 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-

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

The pH of the fermented rumen fluid was estimated immediately after 24 hours of incubation in Hohenheim gas production shaker using Corning electronic pH electrode as per Islam *et al.* (2000). The ammonia nitrogen was estimated by steam distillation as per the protocol of Makkar and Becker (1996).

## 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 by Duncan's multiple range test.

The results pertaining to chemical composition of turmeric expressed on (%) dry matter basis are presented in Table 1. The per cent moisture, CP, CF, EE, TA and NFE content were 14.34, 14.73, 5.42, 2.34, 9.52 and 53.65 per cent respectively. The proximate values obtained in this study are comparable to that observed by Chaturvedi *et al.*, (2016) who reported 13.73, 1.99 and 8.93 per cent CP, EE, and TA, respectively in turmeric. Lower moisture (8.92 %), CP (9.42 %) and TA (2.85 %) content was observed by Ikpeama *et al.* (2014) compared to the values in the present study.

**Table 1:** Chemical composition of turmeric

Parameters	Content (%)
Moisture	14.34±0.28
Crude protein	14.73±0.22
Crude fibre	5.42±0.19
Ether extract	2.34±0.12
Total ash	9.52±0.25
NFE	53.65±0.97

Each value is mean of six observations

The results pertaining to the screening of plant secondary metabolites in turmeric are presented in the Table 2. The phytochemicals identified in the present study is similar to that of presence of alkaloids, saponins, tannins, sterol, phenol and flavonoids observed in aqueous extract (Ikpeama *et al.*, 2014) and in methanolic extract (Rajesh *et al.*, 2013).

**Table 2:** Phytochemical screening of turmeric

Phytochemicals	Tests	Water	Methanol	Ethanol	Chloroform
Alkaloids	Wagner's test	+	+	+	-
Flavonoids	Lead acetate test	+	+	+	+
Tanins	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

In contrast, absence of alkaloids, tannins and saponins in the extracts of turmeric was observed by Arutselvi *et al.* (2012). Absence of phenols and phytosterols in ethanol and chloroform extract in the present study were also observed by Sawant and Godghate (2013). Turmeric contains 3-6% polyphenolic compounds, collectively known as curcuminoids which are responsible for various biological actions (Niranjan and Prakash, 2008). The *in vitro* results of total gas production, methane production, carbon dioxide production, percentage of methane on total gas production, true dry matter digestibility and methane production per 100 mg truly digested substrate after 24 hr incubation of control and turmeric at different dose levels are presented in Table 3. The total gas production of turmeric at 20 mg, 40 mg, 60 mg, 80 mg and 100 mg dose level was 44.33, 43.17, 40.83, 33.67 and 39.50 ml respectively compared to 56.34 ml in control group. The data revealed that significantly ( $P < 0.05$ ) higher total gas production was observed in control group than the turmeric supplemented groups at different dose levels. In contrast,

Al-Hadeethi *et al.*, (2016) observed higher total gas production in turmeric at 10 and 20 g / kg of dry matter compared to control group.

**Table 3:** *In vitro* study of turmeric at different levels

Parameters	Control	20 mg	40 mg	60 mg	80 mg	100 mg
Total gas (ml)	56.34 <sup>c</sup> ±8.36	44.33 <sup>b</sup> ±7.39	43.17 <sup>b</sup> ±2.35	40.83 <sup>ab</sup> ±5.07	33.67 <sup>a</sup> ±8.57	39.50 <sup>ab</sup> ±6.74
Methane production (ml)	12.54 <sup>c</sup> ±1.38	10.00 <sup>b</sup> ±1.94	7.5 <sup>b</sup> ±1.87	6.60 <sup>ab</sup> ±0.816	5.33 <sup>a</sup> ±1.03	5.83 <sup>a</sup> ±0.98
Carbon dioxide production (ml)	45.72 <sup>b</sup> ±8.25	34.50 <sup>a</sup> ±2.42	36.33 <sup>ab</sup> ±4.84	35.16 <sup>a</sup> ±3.18	34.58 <sup>a</sup> ±3.08	32.62 <sup>a</sup> ±3.48
Percentage of methane on total gas production	22.45 <sup>d</sup> ±1.84	19.33 <sup>c</sup> ±1.86	17.33 <sup>bc</sup> ±2.06	16.43 <sup>ab</sup> ±1.62	15.50 <sup>ab</sup> ±3.01	14.33 <sup>a</sup> ±1.03
True dry matter digestibility (%)	62.25 <sup>ab</sup> ±4.86	58.12 <sup>a</sup> ± 4.48	57.64 <sup>a</sup> ±4.07	67.23 <sup>bc</sup> ±5.76	69.50 <sup>c</sup> ±3.79	61.44 <sup>ab</sup> ±5.82
Methane production per 100 mg of truly digested substrate (ml)	9.98 <sup>c</sup> ±1.83	9.22 <sup>c</sup> ±1.44	6.53 <sup>b</sup> ±0.66	5.78 <sup>b</sup> ±0.58	4.34 <sup>a</sup> ±1.14	5.49 <sup>ab</sup> ±1.15
pH	6.67±1.26	6.62±1.35	6.68±1.56	6.57±1.10	6.58±1.47	6.59±1.62
Ammonia nitrogen concentration (mg %)	22.17 <sup>b</sup> ±1.26	21.9 <sup>b</sup> ±2.32	22.53 <sup>b</sup> ±1.47	20.41 <sup>a</sup> ±1.37	20.32 <sup>a</sup> ±1.18	20.45 <sup>a</sup> ±1.63

\*Each value is the mean of six observations; Means bearing different superscripts in a row differ significantly ( $P < 0.05$ )

The methane production (ml) observed were in control group (12.54), turmeric 20 mg (10.00), 40 mg (7.5), 60 mg (6.6), 80 mg (5.33) and 100 mg (5.83) respectively. Similar trend as that of total gas production was observed in methane production also. In contrast to the present study, Al-Hadeethi *et al.*, (2016) reported that addition of turmeric at 10, 20, 30 g / kg of diet dry matter to control diet had no significant effect on rumen methane production in *in vitro* study.

The results of *in vitro* carbon dioxide production (CO<sub>2</sub>) in control, 20 mg, 40 mg, 60 mg, 80 mg, and 100 mg are 45.72, 34.50, 36.33, 35.16, 34.58 and 32.62 ml, respectively. The carbon dioxide production in turmeric was significantly lower ( $P < 0.05$ ) among all dose levels, except 40 mg, compared to control group. The CO<sub>2</sub> production was similar between control and 40 mg dose level. True dry matter digestibility levels were 62.25, 58.12, 57.64, 67.23, 69.50 and 61.44 per cent in control, 20 mg, 40 mg, 60 mg, 80 mg and 100 mg dose level of turmeric respectively. Higher true dry matter digestibility was observed in 60 and 80 mg dose levels while there was no difference among control, 20, 40 and 100 mg dose levels. Al – Hadeethi *et al.* (2016) reported that addition of turmeric at 10 and 20 g / kg of dry matter did not affect ( $P > 0.05$ ) nutrient digestibility. The methane production per 100 mg of truly digested substrate of control and 20 mg, 40 mg, 60 mg, 80 mg and 100 mg dose level of turmeric was 9.98, 9.22, 6.53, 5.78, 4.34, 5.49 ml, respectively. The lowest methane production per 100 mg of truly digested substrate was observed in 80 mg dose level followed by 40, 60 and 100 mg dose levels. Similar results were reported by Pawar *et al.*, (2014) by addition of turmeric oil which reduced the methane production by 15.5 per cent. The highest methane production was recorded in control and 20 mg dose levels. In contrast to the present study, Al – Hadeethi *et al.* (2016) reported that addition of turmeric at 10, 20 and 30 gram/ kg of diet dry matter diet had no significant effect on rumen methane production in *in vitro* study. No difference was observed in pH levels in control and turmeric rhizome supplemented groups. However, significantly ( $p < 0.05$ ) higher ammonia nitrogen concentration was observed in control, turmeric (20 mg) and turmeric (40 mg) supplemented group compared to 60 mg, 80 mg and 100 mg turmeric supplemented groups.

## Conclusion

It could be concluded that turmeric is a good protein source. The highest methane mitigation potential i.e., methane production per 100 mg of truly digested substrate was observed in 80 mg turmeric dose level.

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## Conflict of Interests

There is no conflict of interest.

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