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Effect of Shikakai Pods (*Acacia concinna*) on Phytochemical and Methane Mitigation Potential by *in vitro* Study

R. Balamurugan[1], L. Radhakrishnan[2], R. Karunakaran[1], P. Tensingh Gnanaraj[3], K. Vijayarani[3] 10.18805/ajdfr.DR-1858

ABSTRACT

Background: Several herbs are currently used to mitigate the methane emission in livestock. The shikakai pods (*Acacia concinna*) nutritional composition, phytochemical and methane mitigation potential was evaluated by *in vitro*.

Methods: The crude protein, crude fibre, ether extract, total ash and nitrogen free extract of shikakai pods (*Acacia concinna*) were evaluated. The "phytochemical" screening of shikakai pods (*Acacia concinna)* were qualitatively assessed in water, methanol and ethanol extracts. Further, the different dose levels of shikakai pods at 20, 40, 60, 80 and 100 mg were evaluated *in vitro* for its methane mitigation potential.

Result: The crude protein, crude fibre, ether extract, total ash and nitrogen free extract of Shikakai pods (*Acacia concinna)* were 7.49, 20.81, 1.08, 6.39 and 64.23 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 shikakai pods dose level at 80 and 100 mg was significantly ($p<0.05$) higher than other dose levels.

Key words: Chemical composition, Methane mitigation, Phytochemical, Shikakai.

INTRODUCTION

The scientific name for shikakai pods is *Acacia concinna* and commonly called as "hair fruit". It promotes hair growth, controls dandruff and it reduces hair fall. In ruminants, methanogensis is one of the major reasons for global warming and it decreases the efficiency of nutrient utilization. Enhancing the ruminant performance by altering the rumen microbial eco system and thereby reducing the methane emission is the main aim of ruminant nutritionist. Plant extracts with high concentration of secondary metabolites reduces the methane emission (Teferedegne, 2000). The saponins present in the tropical plants suppress or eliminate the rumen protozoa and reduce the methane and ammonia production (Kamra *et al.,* 2000). Tannins also reduce methane production (Woodward *et al.,* 2001). The pods of shikakai (*Acacia concinna*) also contain saponins. The present study was designed to evaluate *in vitro* the chemical, "phytochemical" and methane mitigation potential of the shikakai pods.

[1]Department of Animal Nutrition, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India.
[2]Central Feed Technology Unit, Tamil Nadu Veterinary and Animal Sciences University, Kattupakkam-603 203, Tamil Nadu, India.
[3]Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India.

Corresponding Author: R. Balamurugan, Department of Animal Nutrition, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai-600 007, Tamil Nadu, India. Email: nutritionbalu@gmail.com

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MATERIALS AND METHODS

The proximate principles of crude protein, ether extract, crude fibre, nitrogen free extract, and total ash of shikakai pods 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 of 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 the method of 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). The above all parameters studied in the Department of Animal Nutrition, Madras Veterinary College, Chennai during the year 2021. The methane concentration and the methane emission in shikakai pods 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 NDS treatment. Methane (ml) per 100 mg of truly digested substrate was calculated based on methane production and *in vitro* dry matter digestibility of substrate incubated using following formula:

Methane production per 100 mg truly digested substrate =

$$\frac{\text{Methane emission (ml)}}{\text{Degradability (\%)}} \times 0.1 \text{ gm}$$

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.

Table 1: Chemical composition of shikakai pods.

Parameters	Content (%)
Crude protein	7.49±0.27
Crude fibre	20.81±0.35
Ether extract	1.08±0.06
Total ash	6.39±0.18
NFE	64.23±1.13

Each value is mean of six observations.

Table 2: "Phytochemical" screening of Shikakai pods.

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 result of six observations.

RESULTS AND DISCUSSION

Chemical composition of shikakai pods (Table 1) contained 7.49, 20.81, 1.08, 6.39 and 64.23 per cent of crude protein, crude fibre, ether extract, total ash and nitrogen free extract respectively on dry matter basis. The CP, EE and TA values observed in this study were higher than that reported by Anamika *et al.* (2017) who reported CP, EE and TA values of 5.28, 0.56 and 4.38 per cent respectively.

The phytochemicals *viz.*, alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids and phyto sterols evaluated in aqueous, methanol and ethanol extracts are presented in Table 2.

The "phytochemical" screening of shikakai pods indicated that alkaloids, flavonoids, tannins, phenols, saponins, carbohydrates, proteins, amino acids and phyto sterols were present in aqueous, methanol and ethanol extracts. Similar presence of alkaloids, flavonoids, tannins, phytosterols and saponins were observed in aqueous, methanol and ethanol extracts of shikakai pods by Todkar *et al.* (2010); Xavier vargeeese raja and Kavithasama, (2012). Komalkhanpara *et al.* (2012) also reported similar presence of saponin, alkaloids, sugar, flavonoids and contrarily, except tannin.

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 evaluated in shikakai pods 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 56.34, 47.50, 48.83, 46, 40.89 and 40.33 ml, respectively. No comparable literature is available for shikakai pods studied at different doses.

The methane production of shikakai pods at 20 mg, 40 mg, 60 mg, 80 mg and 100 mg dose level was 9.33, 9.43, 8.33, 7.33 and 6.50 ml respectively compared to 12.54 ml in control group. The results showed significantly ($p<0.05$) higher methane production in control group than in shikakai pods group. Sirohi *et al.* (2009) reported similar lower

Table 3: *In vitro* study of shikakai pods at different levels.

Parameters	Control	20 mg	40 mg	60 mg	80 mg	100 mg
Total gas (ml)	56.34^{c}±8.36	47.50^{b}± 8.02	48.83^{b}±7.08	46.00^{b}±7.58	40.89^{ab}±6.63	40.33^{a}±11.29
Methane production (ml)	12.54^{d}±1.38	9.33^{c}±1.75	9.43^{c}±2.06	8.33^{bc}±0.82	7.33^{ab}±1.63	6.50^{a}±1.87
Carbon dioxide production (ml)	45.72^{d}±8.25	38.50^{c}±6.62	39.50^{c}±7.06	37.66^{c}±6.43	33.83^{ab}±4.83	33.50^{a}±3.78
Percentage of methane on total gas production	22.45^{c}±1.84	20.16^{b}±2.99	19.50^{b}±2.25	18.43^{ab}±2.06	17.83^{ab}±2.31	16.50^{a}±1.87
True dry matter digestibility (%)	62.25^{ab}±4.86	61.39^{ab}±7.07	64.95^{b}±5.12	59.45^{a}±6.49	57.55^{a}±8.14	57.42^{a}±5.47
Methane production per 100 mg of truly digested substrate (ml)	9.98^{d}±1.83	7.76^{c}±2.08	7.68^{c}±1.01	6.89^{c}±1.15	5.64^{ab}±1.07	4.68^{a}±0.96
Percent of reduction in methane production per 100 mg of truly digested substrate (ml) compared to control	0	22.24^{a}±2.00	23.05^{ab}±1.96	30.96^{c}±1.83	43.49^{d}±1.99	53.11^{e}±2.17

Each value is the mean of six observations.

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

methane production (28.15 ml per gram DM) over control group (37.55 ml per gram DM) in shikakai pods.

The carbon dioxide production (ml) observed in control group (45.72) shikakai pods 20 mg (38.50), 40 mg (39.50), 60 mg (37.64), 80 mg (33.83) and 100 mg (33.50). In this study, carbon dioxide production was significantly ($P<0.05$) lower in different doses of shikakai pods compared to that of control group. The true dry matter digestibility of shikakai pods at 20 mg, 40 mg, 60 mg, 80 mg and 100 mg dose level was 61.39, 64.95, 59.45, 57.55 and 57.42 per cent respectively compared to 62.25 per cent in control group. Significantly higher true dry matter digestibility ($p<0.05$) was observed in control, shikakai pods at dose levels of 20 mg and 40 mg. The results of *in vitro* methane production per 100 mg of truly digested substrate of control, 20 mg, 40 mg, 60 mg, 80 mg and 100 mg are 9.98, 7.76, 7.68, 6.89, 5.64 and 4.68 ml respectively. Significantly ($p<0.05$) 53.1 per cent lower methane production per 100 mg of truly digested substrate was observed in shikakai pods at 100 mg dose level than in the control group.

CONCLUSION

It could be concluded that among different doses studied *in vitro* shikakai pods at 100 mg resulted in lowers (53.11 per cent) methane production per 100 mg truly digested substrate compared to control group.

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Conflict of interest: None.

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