



Efficacy of dietary supplementation of *Tinospora cordifolia* stem in prevention of sub-acute lactic acidosis in goats

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ABSTRACT

Present study was undertaken to investigate the effect of dietary supplementation of *T. cordifolia* for reducing the risk of lactic acidosis in goats. Jamnapari goats (10) were divided into two groups. Goats of treatment group were fed *T. cordifolia* stem powder @ 2% in concentrate feed for 14 days. Control animals were fed equal amount of concentrate without *T. cordifolia* powder. After 14 days, goats were fed overnight soaked wheat grain @ 50 g/kg body weight to induce lactic acidosis. Samples of rumen fluid at 0 and 12 h, and blood were collected on 0, 12 and 36 h of feeding of grain. Lactic acid concentration, total protozoa count and pH of rumen fluid were used as markers to assess the ameliorative potential of *T. cordifolia* stem. Mean lactic acid concentration, protozoa count and ammonia nitrogen of rumen fluid of *T. cordifolia* supplemented goats were 3.47 ± 0.33 mg/dL, $1820.70 \pm 121.36 \times 10^3/\text{mL}$, and 2.71 ± 0.09 mg/dL respectively on 12 h of induction of lactic acidosis. The concentrations of these parameters in control goats were 5.01 ± 0.22 mg/dL, $1168.96 \pm 75.36 \times 10^3/\text{mL}$ and 1.77 ± 0.40 mg/dL respectively. These findings provided evidence that supplementation of *T. cordifolia* stem can prevent lactic acidosis in goats

Key words: Dietary supplementation, Goat, Jamnapari, Lactic acidosis, Rumen fluid, *Tinospora cordifolia*

Lactic acidosis is one of the frequently recorded metabolic disorders particularly in well managed herd which could be acute or sub-acute. Sub-acute lactic acidosis (SARA) is a major nutritional disease occurring in intensive ruminant nutrition due to the allowance of diets rich in highly fermentable ingredients necessary to meet the requirements of the animals. It has been widely described in dairy cows as well as goats (Giger-Reverdin 2018). The recorded incidence of lactic acidosis is up to 40% (Kleen *et al.* 2003) in bovines while in goats up to 18% (Miranda *et al.* 2005). The disease not only causes great economic losses but also animal morbidity. The disease affects rumen micro flora, rumen motility, excessive production of lactic acid, alteration of rumen pH, laminitis, encephalopathy and even death (Radostits *et al.* 2006). Acute form of lactic acidosis is diagnosed frequently and managed with parenteral use of alkalizers (isotonic sodium bicarbonate), antihistamines and thiamine hydrochloride. However, subclinical acidosis many a time remains undiagnosed and cause serious economic losses due to morbidity and reduced production efficiency. Subclinical rumen acidosis is rarely diagnosed as clinical signs are unclear (Enemark and Jorgensen 2001). In ancient times, animals were maintained

by free grazing where plants with medicinal property were also taken by the ruminants while grazing. In modern livestock husbandry, ruminants are mostly stall fed with high grain and low fibre ration which increase the risk of subclinical rumen acidosis (Oetzel 2007).

Medicinal plants have long been used in Indian System of Medicine, viz. Ayurveda for human beings. *Tinospora cordifolia* (Menispermaceae) is a well researched plant for its medicinal properties, viz. disorders of carbohydrate metabolism (Sangeetha *et al.* 2011), antioxidant activity (Khan *et al.* 2011) and immune modulator (Raghu *et al.* 2009). However most of these studies have been conducted either in rats/mice or in human beings. Since the literature on the effect of this medicinal plant on rumen function in ruminant species is meagre, hence present study was conducted to investigate the effects of dietary supplementation of *T. cordifolia* on rumen microflora, pH, lactic acid concentration and organ function in sub-acute rumen acidosis in goats.

MATERIALS AND METHODS

Animal ethical clearance, experimental animals and housing: Adult Jamnapari goats (10) of either sex were used in this study after obtaining permission from Institute Animal Ethics Committee (F1-53/20013/JD-Res). These animals were housed in group and maintained on standard ration and *ad lib.* water. They were acclimatized for 30 days before starting the experiment.

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Source of plant material: Fresh *T. cordifolia* was collected from the natural habitat around Bareilly and identified and authenticated by Dr P. Venu (Scientist – F), Central National Herbarium, Botanical Survey of India, Howrah, India (voucher No CNH/1-1/2013 Tech 11) where voucher reference specimens were deposited. The cylindrical stem of *T. cordifolia* was cut into small pieces (averaging about 2.0 cm in diameter), washed with distilled water to remove the dirt and air-dried. The stems were ground to a coarse powder in a medicinal herb grinding machine, weighed and stored in air tight glass container.

Experimental protocol: The animals were divided into two groups (treatment and control). The animals under treatment group (5 goats) were fed dried *T. cordifolia* stem powder by mixing it in concentrate at dosages of 6% of daily concentrate for 14 days. The rationale for feeding these quantities were based on the doses of methanol extracts of *T. cordifolia* administered to mice in an earlier study (Mathew and Kuttan 1999). The untreated animals (5 goats) were fed equal amount of concentrate without *T. cordifolia* stem powder for similar period.

After 14 days of prophylactic feeding of test herb, overnight soaked whole wheat grain @ 50 g/kg body weight were used for induction of subclinical lactic acidosis in treated group (Kezar and Church 1979). Animal were kept on fasting for 24 h prior to induction of subclinical lactic acidosis. The animals were observed for 72 h after induction to ascertain recovery or treatment as per the condition.

Sampling: Rumen liquor was collected in an anaerobic environment using rumen pump before induction (0 h) and 12 h after induction for estimation of lactic acid, ammonia nitrogen, protozoa count and pH. Blood samples were collected from jugular vein in glass vial EDTA for organ function test at 0, 12, and 36 h after induction of subclinical acidosis. All blood samples were transported to the laboratory in an ice packed container and for estimation of total protein, albumin, total bilirubin, blood urea nitrogen and creatinine in plasma.

Analysis of rumen liquor and blood: Rumen fluid pH was assessed using narrow range pH paper (4.0 to 7.0) with division of 0.3 units (Whatman). Colour, consistency and odour of ruminal liquor were assessed by physical examination of freshly collected rumen liquor. Total count of ruminal protozoa was performed as per the method of Kamra *et al.* (1991). Lactic acid concentration in rumen fluid was estimated following the method of Barker and Summerson (1941). Ammonia nitrogen in rumen fluid was estimated following the method of Weatherburn (1967). The blood samples were then centrifuged to obtain plasma for further estimation of total protein, albumin, total bilirubin, blood urea nitrogen and creatinine using commercial kits (Span Diagnostic, Gujarat, India).

Statistical analysis: Standard error of mean and p-values were used to determine whether there was any significant difference among the groups using Fisher's *t*-test and two-way analysis of variance (ANOVA) following standard protocol (Snedecor and Cochran 1994).

RESULTS AND DISCUSSION

The average pH of ruminal fluid in all the *T. cordifolia* supplemented goats were in range of 5.8 when measured 12 h after the experimental induction while the untreated group showed the average pH 5.2. In comparison to untreated goats, *T. cordifolia* supplemented goats had significantly ($P \leq 0.05$) lower lactic acid concentration in rumen liquor (Table 1). *T. cordifolia* supplementation also significantly ($P \leq 0.05$) increased the protozoa counts of rumen liquor (Table 1). After excess grain intake, the change in colour, consistency and odour of ruminal fluid were milder in *T. cordifolia* supplemented goats than that of untreated goats. The faecal consistency was loose but no diarrhea was seen in animals supplemented with *T. cordifolia* during entire observation period whereas mild diarrhea was noticed in untreated group. The liver function of goats of both the groups was unaffected as evidenced from concentrations of total protein, albumin and total bilirubin measured as at 0, 12 and 36 h after intake of excess grain (Table 2). Similarly, the renal function tests, blood urea nitrogen and creatinine did not differ significantly ($P \leq 0.05$) between untreated control and treated group.

Sub-acute lactic acidosis is a digestive disorder that primarily affects high yielding dairy cows and feedlot cattle leading to negative impact on health and productivity. High energy and low fibre diet although increases the energy availability for enhanced production but these diets also predisposes for development of subacute ruminal acidosis (Bipin *et al.* 2016). In field conditions, subclinical cases of lactic acidosis are often undiagnosed and do not get proper treatment as the clinical signs are vague and difficulties in analysis of rumen fluid in Indian field conditions. Prolong subclinical lactic acidosis may cause ruminitis, ruminal abscess, laminitis, reduced fertility and decreased

Table 1. Total protozoa counts, ammonia nitrogen and lactic acid content (Mean $\pm$ SEM) in rumen fluid of goats

Parameter		Mean $\pm$ SEM		P value*	
		<i>T. cordifolia</i> supplemented (Treatment)	Control	Treatment Interval	
Total	0 h	1125.18 $\pm$ 55.19	1201.19 $\pm$ 109.60	0.03	0.04
protozoa count (10 ³ /mL)	12 h	1820.70 $\pm$ 121.36	1168.96 $\pm$ 75.36		
Ammonia nitrogen (mg/dL)	0 h	2.39 $\pm$ 0.14	2.36 $\pm$ 0.16	0.52	0.82
	12 h	2.71 $\pm$ 0.09	1.77 $\pm$ 0.40		
Lactic acid (mg/dL)	0 h	3.04 $\pm$ 0.08	4.63 $\pm$ 0.03	0.01	0.03
	12 h	3.47 $\pm$ 0.33	5.01 $\pm$ 0.22		

* $P < 0.05$ differs significantly.

Table 2. Plasma biochemical changes (Mean±SEM) in goats with lactic acidosis

Parameter	Mean±SEM		P value*	
	<i>T. cordifolia</i> supplemented (Treatment)	Control	Treatment	Interval
Total protein (g/dL)	0 h 6.76±0.24 12 h 6.85±0.41 36 h 6.81±0.46	6.47±0.43 6.68±0.37 6.88±0.29	0.35	0.38
Albumin (g/dL)	0 h 2.34±0.26 12 h 2.87±0.33 36 h 2.85±0.06	2.59±0.25 2.69±0.43 2.77±0.24	0.97	0.26
Total bilirubin (mg/dL)	0 h 0.20±0.06 12 h 0.34±0.03 36 h 0.26±0.06	0.17±0.03 0.38±0.05 0.35±0.10	0.94	0.21
BUN (mg/dL)	0 h 4.13±0.34 12 h 3.74±0.42 36 h 4.27±0.55	3.33±0.34 3.53±0.30 2.97±0.44	0.44	0.79
Creatinine (mg/dL)	0 h 1.55±0.28 12 h 1.25±0.07 36 h 1.33±0.26	1.50±0.24 1.55±0.15 1.07±0.16	0.98	0.42

*P<0.05 differs significantly.

production (Oetzel 2007). The thresholds of ruminal pH for the diagnosis of SARA have been set 5.6–5.8 (Khafipour *et al.* 2009). Consistently, ruminal pH value less than 5.8 lasted more than 3 h was considered as the critical value of SARA diagnosis.

Considering the clinical importance of problem, the effect of *T. cordifolia* supplementation on rumen function was assessed on the basis of its effect on total protozoa count, lactic acid concentration, and pH after induction of subclinical acidosis. The results of our study show that *T. cordifolia* supplementation was capable of buffering the effect of increased concentration of lactic acid in rumen fluid. It also protected the rumen protozoa and brought spontaneous recovery of all the animal without multi system complications. SARA is known to be associated with inflammations of different organs and tissues. The clinical signs in SARA could be attributed to pathophysiological cascade in rumen. The inflamed ruminal epithelium could provide seat for colonization of bacterial which may gain access to portal circulation leading to liver abscesses and peritonitis. Besides, bacteria may also colonize lungs, heart valves, kidneys or joints leading to pneumonia, endocarditis, pyelonephritis and arthritis (Oetzel 2007). Therefore, liver and kidney function test could be useful in monitoring the progression of SARA. The possible reason for unaltered organ function test in present study could be attributed to the short period of exposure to grain; however the prolonged exposure to high concentrate diet resulted in decline in milk quality and activation of the acute phase response, inflammatory responses and cortisol (Jia *et al.* 2014).

T. cordifolia has been used traditionally in India and elsewhere for varied purposes such as general tonic, anti-inflammatory, hepatoprotective, anti-allergic, anti-diabetic

and immunomodulator. However, there is no published report of effect of *T. cordifolia* on subclinical lactic acidosis therefore our data could not be compared. The stem of *T. cordifolia* is rich in alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoid, phenolics, aliphatic compounds and polysaccharides. The active ingredients induce stimulation on the salivary gland, gastric juice and improve digestive function (Sengupta *et al.* 2013).

The stem of *T. cordifolia* is reported to contain chemical constituents including tinocordifolin, tinosporone, tinocordioside, cordioside, picrotine, colombine and columbin (Singh *et al.* 2003). The exact mechanism by which *T. cordifolia* prevents subclinical lactic acidosis is not yet clear but the presence of glycoside and polysaccharides has been reported in stem of *T. cordifolia*. These chemical constituents contain -OH group in their structure which possibly interacts with lactic acid and helps in restoring healthy rumen microflora and rumen liquor. In addition, presence of glucosidal bitter principle in the stem of *T. cordifolia* may stimulate salivary gland and thus increase the secretion of saliva juice containing alkaline bicarbonate which may have beneficial effect on buffering lactic acid in rumen fluid as well as in improving whole digestive system.

Supplementation of *T. cordifolia* prevents development of subclinical lactic acidosis in goats by maintaining rumen pH, protozoa count and lactic acid concentration. However, further studies are required to establish the mechanism of interference and activity guided separation and characterization of compounds in *T. cordifolia* stem involved in restoration of rumen function.

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