ESTIMATION OF TOTAL SAPONIN CONTENT OF COMMONLY AVAILABLE FODDER CROPS FOR DAIRY CATTLE

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Saponin is one of the important plant secondary metabolite which has been shown to selectively modulate the rumen microbial populations resulting in an improvement of rumen fermentation and decreases the methane production. Chemically saponins are high molecular weight glycosides in which a triterpene saponins or steroidal aglycone moiety is linked to one or more sugar chains. The saponins may be considered a part of plant defense system because they are known to inhibit mould, to be antimicrobial and to protect plants from insect attacks (Francis *et al*., 2002). Saponin in higher level causes bloat and lower level beneficial for ruminants. The majority of research on saponin has been employed to exploit it for inhibition of rumen ciliate protozoa and selectively inhibiting some bacteria, which might reduces the production of hydrogen ions for the methane emission (Bharathidhasan *et al*., 2013). Currently numerous studies had been attempted to exploit these saponin as a natural feed additive to modulate the efficiency of rumen fermentation by enhancing protein metabolism, reducing nutritional stress or bloat, decreasing methane emission and improve the animal health and productivity (Patra *et al*., 2006). Early study has been reported that plants rich in saponins decreased the methane production in the rumen (Agarwal *et al*., 2006). Similarly Goel *et al*. (2008) observed that the methane emission was decreased by the way of decreasing the methanogen populations through *Sesbania saponins* by 78 %, *Fenugreek saponins* by 22 % and *Knautia saponins* by 21% by *in vitro* fermentation technique. Saponin extracts from *Yucca schidigera* and *Quillaja saponaria* had been demonstrated to reduce methanogenesis by *in vitro* (Holtshausen *et al*., 2009). Sterol binding capability of saponins has been implicated to the destruction of protozoal cell membranes (Hostettmann and Marston, 1995). The saponin appears to reduce methane emission by inhibiting protozoa and interfering hydrogen transfer between the protozoa and associated methanogens. The decrease in methane emission will reflect on increasing the production performance of dairy cattle. Hence the present study was carried out with an objective to find out the total saponin content of the commonly available fodder crops for dairy cattle.

Six samples each of sixteen commonly available forages in Tamil Nadu used for feeding dairy cattle were collected (approximately 2 kg on each sample) as per the standard procedure. The collected forage samples were dried in a hot air oven at a

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temperature of 55 - 65˚C to constant weight and ground to pass through 1 mm sieve and stored in airtight containers for saponin analysis. The plant extracts were prepared (Sirohi *et al*., 2009) by weighing of one gram sample into 250 ml conical flask and to it 10 ml of 50 % aqueous methanol was added and the flasks were tightly sealed and kept in a orbital shaker at 120 rpm for 24 hrs. After which the contents of the flask was filtered through four layers of muslin cloth. The filtrate obtained was further filtered through Whatman no.1 filter paper and the resultant filtrate was used for total saponin estimation. Saponin in plant extracts were determined using colorimetric method described by Baccou *et al*. (1977). In a test tube containing 0.25 ml of plant extract,1.75 ml ethyl acetate and 1.0 ml of reagent A (0.5 ml anisaldehyde + 99.5 ml ethyl acetate) and 1.0 ml of reagent B (50 ml concentrated sulphuric acid + 50 ml ethyl acetate) were added and mixed thoroughly and incubated in a water bath maintained at 60° C for 20 minutes. After cooling to room temperature the optical density was measured at 430 nm in a UV spectrophotometer (Perkin Elmer model Lambda 25). A reagent blank was also simultaneously measured for its optical density. The concentration of saponin in the plant extract was calculated by fitting the optical density values in saponin standard curve. Saponin standard curve was drawn by determining the optical densities of standard saponin solutions of varying strength (0.1 mg to 1.0 mg) adopting the same procedure as that for plant extracts. The data were statistically analysed through the procedure of statistical analysis system (IBM SPSS® Version 20.0 for Windows®) as per the Snedecor and Cochran (1989).

A highly significant ($p<0.01$) difference was observed between grass/shrubs and tree fodders in total saponin levels (Table 1). Among the grass/shrubs *Vigna unguiculata* had maximum level (3.80 %) of total saponin and the minimum saponin level (0.82 %) was observed in the *Brachiaria mutica.* The total saponin content of the other forages like *Stylosanthus scabra, Sorghum vulgare, Saccharum officinarum, Medicago sativa, Pannicum maximum, Cyanodon dactylon and Pennisetum typhoides x Pennisetum purpureum were 3.70 %, 2.87 %, 1.72 %, 1.53 %, 1.49 %, 1.22 % and 0.84 %* respectively. The present findings on total saponin of the forages were almost consistent with that reported earlier. The *Medicago sativa* contained similar saponin level (1.72 %) as that reported (0.78 - 1.78 %) by Vieira and Borba (2011). The range of saponin in other grasses was little higher than the observed values recorded in this experiment. In the tree fodders, *Acacia nilotica* (6.18 %) had highest total saponin content and *Gliricidia sepium* (3.48 %) had lowest total saponin content. The total saponin content of other tree fodders like *Albezia lebback, Moringa oleifera, Sesbania grandiflora, Leucaena leucocephala and Azadirachta indica* was 3.68 %, 3.86 %, 3.98 %, 4.13 % and 4.16 % respectively. It was concluded that the highest total saponin was presented in *Acacia nilotica* (6.18 %) and *Vigna unguiculata* (3.8 %) among tree fodders and grass/shrubs groups respectivley.

The total saponin content of *Acacia nilotica* (7.36 %) and *Leucaena leucocephala* (5 %) reported by Almahy and Nasir (2011) and Belewu *et al*. (2008) respectively were higher than the present findings. The total saponin in *Azadirachta indica* (4.16 %) was higher in the present study than the earlier reported values of 3.21 % by Sirohi *et al*. (2009). A minimum saponin content of 1.6 % was reported by Ogbe *et al*. (2011/12) and maximum content of 4.09 % was reported by Alexander *et al*. (2008) in *Moringa oleifera.* There are some factors, such as physiological age, environmental and agronomic factors, which may affect the saponin content of plants (Yoshiki *et al*., 1998).

The present study revealed that the tree fodders had higher level of saponin than grasses. *Acacia nilotica* contained highest level of saponin. The variations in the concentration of total saponin between the present study and reported values might be due to the maturation of plants because of the physiological changes occurring during the plant growing cycle, soil type, fertility, water supply are known to affect the concentration in plants. Moreover species vary in their response to climatic and physiological changes that induces the changes in chemical composition and in particular, in the concentration of secondary compounds (Dann and Low, 1988). Saponin supplementation through forages has been employed to exploit it for inhibition of rumen ciliate protozoa which might improve the efficiency of microbial protein synthesis by reducing microbial protein turn over and enhance protein flow to the duodenum and ruminant production (Patra and Saxena, 2009). Currently numerous studies had been attempted to exploit these saponin as a natural feed additive to modulate the efficiency of rumen fermentation by enhancing protein metabolism, reducing nutritional stress or bloat, decreasing methane emission and improve the animal health and productivity (Patra *et al*., 2006). Agarwal *et al*. (2006) observed that methane production was reduced by *in vitro* with water, ethanol and methanol extracts of soap nut (*Sapindus mukorossi*). Addition of Tea saponin at 2, 4, 6 and 8 mg against 200 mg mixture *in vitro* decreased methane concentration by 13, 22, 25 and 26%, respectively (Hu *et al*., 2005). Hence, the present study might be helpful for the inclusion of Saponin rich forages in ruminant diet to improve the rumen fermentation and reduce the methane emission to environment.

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Table 1.Total saponin content of commonly available fodder crops for dairy cattle on DM basis (Mean# ± S.E)

S.No.	*Forages*	Total Saponin
Grasses/Shrubs		
1	*Pannicum maximum*	1.49 ± 0.18
2	*Cyanodon dactylon*	1.22 ± 0.07
3	*Pennisetum typhoides x Pennisetum purpureum*	0.84 ± 0.16
4	*Brachiaria mutica*	0.82 ± 0.03
5	*Sorghum vulgare*	2.87 ± 0.35
6	*Medicago sativa*	1.72 ± 0.39
7	*Vigna unguiculata*	3.80 ± 0.48
8	*Stylosanthus scabra*	3.70 ± 0.32
9	*Saccharum officinarum*	1.53 ± 0.17
Tree fodders		
10	*Gliricidia sepium*	3.48 ± 0.25
11	*Leucaena leucocephala*	4.13 ± 0.55
12	*Sesbania grandiflora*	3.98 ± 0.57
13	*Acacia nilotica*	6.18 ± 0.25
14	*Albezia lebback*	3.68 ± 0.32
15	*Azadirachta indica*	4.16 ± 0.38
16	*Moringa oleifera*	3.86 ± 0.11
Statistical significance between Grasses/shrubs and Tree fodders		
	Grasses/Shrubs* (Mean ± S.E)	2.66 ± 0.23[a]
	Tree fodders* (Mean ± S.E)	4.03 ± 0.18[b]
	Test of significance	$p<0.01$

Mean of six observations

* Means bearing different superscripts in the same column differ significantly