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Potential of Some Legume Forages for Rumen Defaunation in Goats

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ABSTRACT

Plant secondary metabolites appeared to have some effects on rumen fermentation pattern, microbial population, feed intake and digestibility. An experiment was conducted to identify forage species with potential in defaunating (killing protozoal population) the reticulo-rumen in goats. There were five forage species tested: Robles (*Cassia siamea),* Desmodium (*Desmodium heterophyllum)*, Kakawate *(Gliricidia sepium*), Centro (*Centrosema pubescens)* and Ipil-ipil *(Leucaena leucocephala)* with sodium lauryl sulfate (SLS) as the control treatment. The forages of the different species were homogenized using a blender to extract the juice. The extracts were then drenched down into the reticulo-rumen of goats through stomach tubing at 1% of their body weight (BW).

Froth analysis for saponin content showed higher values in Robles followed by Ipil-ipil, with Kakawate as the lowest. Ipil-ipil and Robles had comparable defaunating effects with that of the SLS which significantly reduced ($p<0.01$) the protozoal numbers as compared to the other forages tested. However, Ipil-ipil and Desmodium appeared to have significantly higher ($p<0.01$) dry matter (DM) intake of the basal Napier grass (*Pennisetum purpureum*) diet as compared to the other forages tested. Bacterial population also decreased but differences among treatments were not significant. Therefore, forage extracts containing high amounts of saponin are effective in reducing protozoal population, comparable to that of SLS. The use of Ipil-ipil forage extract for defaunating the rumen is recommended as it is organic, unlike SLS, and also promotes higher dietary DM intake.

Keywords: Rumen defaunation, saponin-containing forage extracts, goats

INTRODUCTION

One of the restrictions on the use of tropical forages as livestock feed include low levels of good quality protein or nitrogen, less fermentable carbohydrates and lipids, and low digestibility because of high fiber content (Diaz *et al.,* 1993; Wanapat, 2000; Ngamsaeng *et al.,* 2006). These result to poor animal performance because of decreased voluntary feed intake (VFI) and an imbalance in the absorbed nutrients (protein-to-energy ratio), causing slow growth, and low reproductive rate and milk production performance (Diaz *et al.,* 1993). Such limitations are aggravated by the presence of anti-nutritional factors which may

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also affect the voluntary intake of the animals as well as feed digestibility so that some plants are less accepted or even rejected (Ba and Ngoan, 2003). These anti-nutritional compounds such as tannins, lignins, saponins, and mimosine are assumed to be synthesized in plants to protect them against invading microbes and insects. When consumed by the animals, these compounds limit the growth of different types of microbes (useful and undesirable) in the rumen as quality of roughage exhibits close relationship with rumen ecology, microbes, and fermentation patterns (Wanapat, 2000; Kamra, 2005).

The effects of these plant secondary metabolites may appear detrimental in animal nutrition, but they can also be capitalized to manipulate rumen function and fermentation (Ba and Ngoan, 2003; Fulgueira et.al, 2007 and Leng *et al.,* 2011). As nutrient requirements of ruminants depend largely on rumen microorganisms, their population dynamics is affected by diet changes. Though protozoa contribute to fiber digestion, it also posts negative effects on bacteria as protozoa prey on bacteria especially when dietary protein is lacking. This is disadvantageous to the host animal fed with low-protein, highly fibrous diets, so that eliminating the protozoa or defaunation increases the amount of microbial protein and dietary protein available to the host animal (Leng *et al.,* 2011), thus increasing the absorbed nutrients (Gebeyehu and Mekasha, 2013).

The compounds known to defaunate the rumen include saponins and tannins and they are reported to be prevalent in many tropical fodder plants (Makkar *et al.,* 1995; Pell *et al.,* 2001 and Leng, *et al.,* 2011). Numerous studies have now proved that tannins and saponins have toxic effects on protozoa (Diaz *et al.,* 1993; Monforte-Briceno *et al.,* 2005), and the use of some tree leaves or plant extracts (which are organic compared to sodium lauryl sulphate) reduced the number of rumen protozoa and improved intake, fiber digestion, and weight gain (Eugene *et al.,* 2004; Ozdemir *et al.,* 2006; Patra and Saxena, 2009 as cited by Lopez-Camarena, *et al.*, 2010).

The use of plant extracts as defaunating agent is supported by Patra *et al.* (2006). Forages of *Centrosema pubescens, Desmodium intortum*, *Leucaena leucocephala*, *Vigna parteri, Desmodium uncinatum* as defaunating agents were tested *in vitro* but *Enterolobium cyclocarpum* was found to be effective *in vitro* and *in vivo* (Leng *et al.,* 2011). There is, therefore, a dearth of information on the defaunation potential of fodder trees and other locally-growing forage species, hence, this study.

MATERIALS AND METHODS

Preparation of Treatment Forages

The following forages were tested as defaunating agents: (1) Robles (*Cassia siamea)* leaf extract; (2) Desmodium *(Desmodium heterophylum)* leaf extract, (3) Kakawate *(Gliricidia sepium)* leaf extract, (4) Centro (*Centrosema pubescens)* leaf extract, (5), Ipil-ipil *(Leucaena leucocephala)* leaf extract, and (6) the use of detergent sodium lauryl sulfate (SLS) served as the control treatment.

The fresh leaves of the plant species were weighed and washed using tap water, then drained and ground in a blender to obtain a finer particle size for easy squeezing of the extract. Tap water was then added at 1:1 ratio (1kg of fresh leaves: 1 liter of tap water) similar to the procedure used by Valleser (2011) and Aban (Figure 1).

At least 1000 ml of leaf extracts was collected from each plant species which was analyzed for pH content using a digital pH meter. The juice was either given fresh or stored in the refrigerator at 4°C for not more than 24 hours after extraction before giving to the animals.

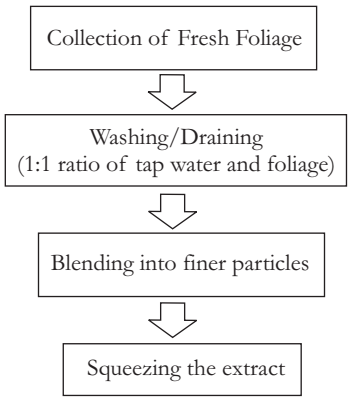

Figure 1. Preparation of extracts from test forages.

Experimental Design

The experiment was set-up following RCBD using sex/age of the experimental animals and period of conduct as bases for blocking, with four blocks per treatment.

Preparation and Feeding of Test Animals

The experimental animals were confined individually to avoid direct contact with pastures and soil, and with other animals (Kiran and Mutsvangwa, 2001; Ozdemir, *et al.,* 2006) for possible re-infection after treatment. The cages were cleaned and disinfected before placing the animals, and goats, aging 4-6 months and weighing 10-15 kg were dewormed before the conduct of the study.

The experimental goats were fed twice a day (8:00 AM and 4:00 PM) of a ration comprising a basal diet of Napier grass (*Pennisetum purpureum*) and the extracts of treatment forages as supplement.

Laboratory Analyses

Basal diet which is Napier grass was analyzed for its DM content using convection oven set at 100C for about 24 hours (Bestil, 2009) to be used in determining the feed requirement of the experimental animals, and in computing the dry matter intake of animals.

Rumen Defaunation of Animals

An adjustment period of six (6) days was observed when animals were given the basal diet of Napier grass at *ad libitum* to eliminate the previous diet and get the

animals conditioned to the experimental set-up. The rumen fluid of the animals was also examined every three days to monitor changes in protozoal numbers (Ushida *et al.,* 1990).

Targeting to only partially defaunate the rumen, feed was not totally withdrawn during the defaunation period (Han *et al.,* 1999; Kiran and Mutsvangwa, 2010). Administration of the defaunating agents was based on the method used by Dundar (2004) and Valleser(2011) with the following modifications:

Day 1 - the feed was reduced into half of *ad libitum* intake, while a rumen fluid was collected for the initial measures of pH, bacterial and protozoal counts.

Day 2- 4 - still being fed at 50% of *ad libitum* intake, the defaunating agents were administered every day in three (3) consecutive days by drenching. The leaf extracts were administered to the animals at 1% of body weight, similar to the amount used by Chauhan *et al.* (2004) and Valleser (2011). Two (2) hours post-dosing in Day 3, the animals received a substrate solution of flour and sugar to sustain the bacterial population in the rumen when the animals were fed half full (Valleser 2011).

Day 5 - feeding the animals back into *ad libitum* of the basal Napier grass.

The defaunating agents were administered directly into the reticulo-rumen through a rubberized stomach tubing inserted into the esophagus down to the reticulo-rumen similar to collecting rumen fluid (Ozdemir *et al.,* 2006). For the control treatment (using SLS), 0.8 gram powder was dissolved in 10 ml water/ kg body weight (BW), similar to that used by Santra and Karim (2000) and Valleser and Bestil (2011).

Rumen Fluid Collection and pH Measurement

Rumen fluid was collected via stomach tube and strained through four (4) layers of cheese cloth (Ozdemir, *et al.,* 2006). A volume of about 8-10 ml rumen fluid was collected one day before the administration of the defaunating agents and the fluid was examined for initial rumen pH, protozoal and bacterial counts.

The second collection of rumen fluid was done on the fourth (4^{th}) day, two hours after administering the last dose of the defaunating agent. Last collection was done on the seventh (7^{th}) day, allowing enough period for the animals to fully recover their appetites after the defaunation treatment. This method is supported by the study of Valleser and Bestil (2011) which showed that the rumen has stabilized on the 9^{th} day after defaunation. The rumen fluid from second and last collections was then subjected to pH analysis and microbial counting and the results served as final reading (Eugene *et al.,* 2004).

Protozoal Counting Procedure

The collected rumen fluid was immediately placed in a test tube and was serially diluted into 1:10 dilutions similar to the procedure used by Rowe, *et al.* (1985) as cited by Valleser (2011). Logul's solution was added into the rumen fluid to stabilize the protozoa (Eugene *et al.,* 2004). Protozoal counting was done under a microscope using 1000x objective lens following the method used by Valleser (2011). Protozoal count was expressed as cell counts per ml of rumen fluid:

Protozoal Cell counts/ml = Number of protozoal cells x 1000 magnification x dilution rate.

Bacterial Counting Procedure

The rumen fluid was serially diluted into 1:10 up to 1: 1 million dilutions and 1 ml representative drop was poured into a petri plate with a prepared medium of plate count agar. It was then incubated for 18–24 hours. After incubation, the bacterial colonies were counted using a colony counter. The counted colonies were expressed as colony forming units/ml rumen fluid, as follows:

Colony forming units (cfu/ml) = Number of bacterial colonies x dilution rate

Data Gathered

1. pH of plant extracts
2. Ruminal pH of goats
3. Protozoal count (cell count/ml rumen fluid)
4. Bacterial count (cfu/ml rumen fluid)
5. Dry Matter Intake (DMI)

DMI, kg = [(Feed given × DM of given) – (Feed refused × DM of refused)]

6. Dry Matter Intake (% Body Weight)

This was computed to account the variations in body size affecting voluntary DMI, as follows:

$$\text{DMI, BW} = \frac{\text{Dry Matter Intake, kg}}{\text{Live Weight, kg}} \times 100$$

7. Saponin and Tanin Analysis of plant elements

Saponin analysis was conducted following the method used by Guevara *et al.* (2005) in froth test analysis. Measurement in the rise of honeycomb froth (cm) was considered as quantitative result of the analysis done at the Department of Pure and Applied Chemistry, VSU. Tannin analysis was conducted using the method of Guevara et al. (2005).

Analysis of Data

Data were analyzed using general linear model (GLM) for two-way univariate analysis of variance, and comparison of treatment means was done by Honestly Significant Difference (HSD) test using the Statistical Package for Social Sciences (SPSS) ver. 17.0 software.

RESULTS AND DISCUSSION

Defaunating Characteristics of Test Forages

Secondary metabolites present in forages are believed to show defaunating activity in the ruminant's stomach. The characterization of the forages tested is presented in Table 1. Presence of the phytochemicals (tannin and saponin) was determined following the method used by Guevarra *et al.* (2005). Froth test analysis for saponin using fresh sample showed that all forages tested, except for Kakawate, were positive for saponin and only Centrosema appeared to be positive in tannin. The pH values range from 4.2 to 8.0 and showed significant differences among forages tested.

Table 1. Contents of saponin and tannin and pH values of forage defaunating agents tested.

Treatments	Saponin Content (Froth test method)*		Tannin Content*	pH content
T_0 – SLS				8.0^a
T_1- Robles	+	3.5cm	-	4.2^d
T_2 - Desmodium	+	2.5cm	-	6.0^c
T_3 - Kakawate	-	0.3cm	-	7.1^b
T_4 - Centrosema	+	3.0cm	+	5.6^c
T_5 - Ipil-ipil	+	3.1cm	-	6.0^c
p - value				*0.001*

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**Analyzed by the method of Guevara et al. (2005).*

Changes in Protozoal and Bacterial Population

While initial protozoal counts among experimental animals did not have significant differences, post treatment protozoal counts differ significantly, indicating that some of the forages tested are more potent than others and *Leucaena leucocephala* (Ipil-ipil) extract was even observed to be comparable to sodium lauryl sulfate (SLS) detergent (Table 2 and Figure 2).

Bacterial count also decreased with the administration of the plant extracts. There were no significant differences in bacterial count observed post treatment, indicating similar effects of the forage extracts tested on bacterial population as that of the control (SLS). It should also be noted that SLS treatment (control) had the greatest reduction not only in protozoa but also in bacterial count which imply decreased fiber digestion of the basal forage diet.

Table 2. Bacterial and protozoal counts before and after administration of the forage extracts as defaunation agents.

Treatments	Protozoal Count (cell count/ml)			Bacterial Count (cfu/ml)		
	Initial $1x10^4$	Final $1x10^4$	% Decrease in Protozoa	Initial $1x10^8$	Final $1x10^8$	% Decrease in Bacteria
T_0 - SLS	61.1	1.1^a	98.21^a	11.42	4.4	62.39
T_1- Robles	60.5	7.3^b	87.97^b	16.9	7.0	58.73
T_2 - Desmodium	55.8	15.1^c	72.65^c	14.95	6.6	55.45
T_3 - Kakawate	55.6	24.0^d	56.32^d	14.4	6.6	54.09
T_4 - Centrosema	54.7	34.0^e	40.03^e	16.8	6.9	58.99
T_5 - Ipil-ipil	58.7	3.8^{ab}	93.47^{ab}	16.9	7.1	56.90
p - value	*0.260ns*	*0.001***	*0.001***	*0.569ns*	*0.839ns*	*0.302ns*

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ns Not significant; **Highly significant

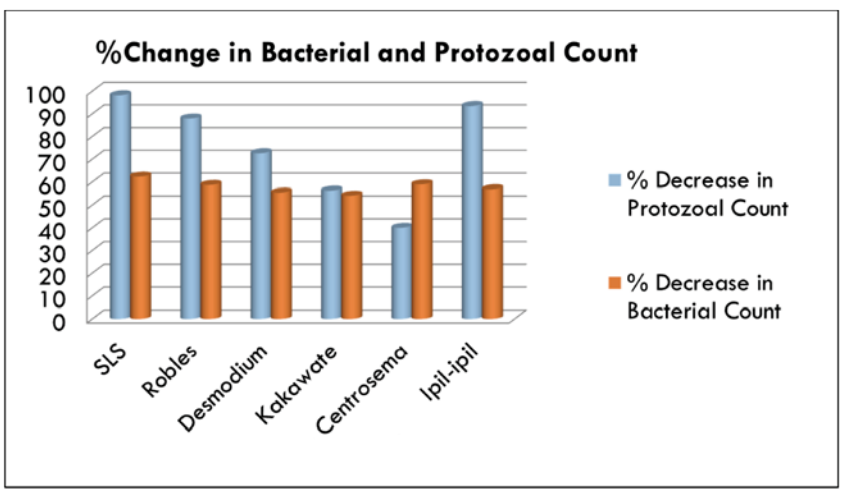

Figure 2. Percent change in protozoal and bacterial counts after the administration of forage extracts as defauating agent.

Ipil-ipil showed to be the best defaunating agent among the forages tested as it exhibited the greatest reduction in protozoal numbers. This reduction in protozoal numbers could be due to the inhibitory effects of phenolic compounds of the plant extract such as saponins, where results of previous studies conducted provided support (Busquet *et al.,* 2006; Lila *et al.,* 2003). According to Teferedgne (2000) and Babayemi *et al.* (2004), feedstuffs containing saponin had shown to defaunate the rumen and reduce methane production. The decline in protozoal counts with the administration of saponin-rich forage extracts could be due to the binding of saponin with the sterol of protozoal cell membrane which alters cell wall permeability (Newbold et al., 1997). The antiprotozoal effect of saponins can also be due to their capacity to form irreversible complexes with cholesterol in the protozoal cell membrane, causing breakdown of the membrane, cell lysis, and death (Francis *et al.,* 2002). According to Moss *et al.,* (2000), all rumen microbes including protozoa are vulnerable to saponin-induced changes in cell membrane properties.

While Centrosema appeared to contain tannin, it had the lowest percent reduction in protozoal numbers among the forages tested. Abarghuei *et al.,* (2010) reported that tannins and secondary metabolites reduced protozoal numbers, and has inhibitory effects on feed digestion, microbial population and enzyme activity in many experiments (Patra *et al.,* 2006). Ruiz (2014) and Busquet (2006) added, however, that no definite explanation could be established from comparing studies about the effects of tannins on protozoal population in the rumen due to variations in the type of diet, phenolic level, species, individual animal differences, the dose used, and sampling methods.

Williams (1991) present positive effects of reducing the concentration of rumen protozoa as it could increase microbial protein production and amino acids available for absorption, and a decline in protozoal numbers is linked with increasing propionate. Poungchompu *et al.* (2009) stated that populations of protozoa and fungi dramatically reduced when dairy heifers were fed with plants containing condensed tannins and saponins.

Changes in rumen pH

Maintaining a slightly acidic to neutral rumen pH of ruminant animals is important in order to achieve stability of the rumen environment. As presented in Table 3, results showed that rumen pH is significantly affected by the administration of the defaunating agents. While rumen pH levels before administration of the extracts were normal and more or less similar among experimental animals, significant differences in rumen pH were observed after the defaunation procedure (day 4 and 7). It was observed that there was a dramatic decrease in rumen pH among treatments, except T_0, in day 4 which was measured two (2) hours after the last dose of the defaunating agents. The decrease in pH ranges from 9.79 to 32.46%, apparently affected by the pH of the defaunating agents. A similar observation was noted in day 7 where rumen pH of T_1 up to T_5 groups dropped while that in T_0 increased. Rumen pH readings of T_1 up to T_5 groups where not significantly different ($p<0.01$) from each other, and the percent reduction ranged only from 5.42 to 10.13% as compared to that observed in day 4.

Table 3. Changes in rumen pH with the administration of the forage extracts and their effects on protozoal population.

Treatments	pH of Defaunating agents	Initial Rumen pH (Day 0)	Mid Period (Day 4)		Final Period (Day7)		% Decrease in Protozoal Count
			Rumen pH	% Change in Rumen pH	Rumen pH	% Change in Rumen pH	
SLS	8.0^{a}	7.24	8.18^{a}	13.08^{c}	7.45^{a}	2.91^{b}	98.21^{a}
Robles	4.2d	7.25	4.90^{d}	-32.46^{a}	6.68^{b}	-7.95^{a}	87.97^{b}
Desmodium	6.0^{c}	7.28	5.93^{ab}	-25.10^{b}	6.78^{b}	-6.82^{a}	72.65^{c}
Kakawate	7.1^{b}	7.32	6.60^{b}	-9.79^{b}	6.90^{b}	-5.73^{a}	56.32^{d}
Centrosema	5.6^{c}	7.22	5.78^{c}	-20.00^{ab}	6.83^{b}	-5.42^{a}	37.80^{e}
Ipil-ipil	6.0^{c}	7.32	6.0^{ab}	-27.92^{b}	6.58^{b}	-10.13^{a}	93.47^{ab}
p - value	*0.001*	*0.749*	*0.001*	*0.001*	*0.001*	*0.001*	*0.001*

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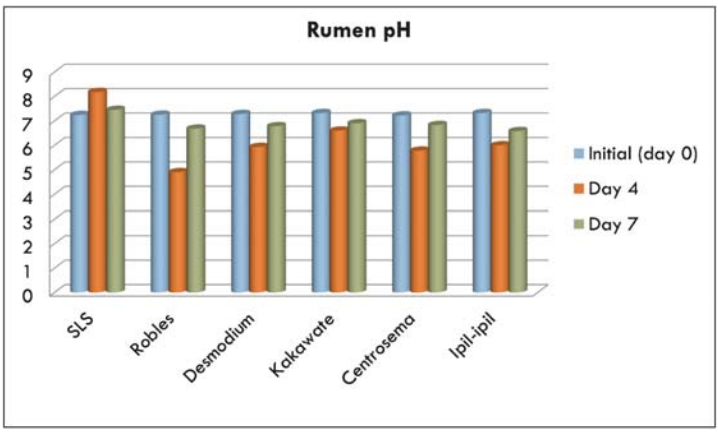

Figure 3. Fluctuation in rumen pH with administration of forage extracts affecting protozoal population.

According to Franzolin *et al.* (2010), rumen pH can vary from 5.5 to 7.5 depending on the type of diet and the feeding frequency, and Clarke (1977) reported that increases in rumen pH beyond 7.8 and a decrease to 5.0 are detrimental to rumen ciliate protozoa because of their sensitivity to adverse rumen pH changes. The great reduction in protozoal numbers in this study could also be due to the lower pH of the forage extracts used, and the reduction in rumen pH may not have a similar pattern of differences as that of the reduction in protozoal numbers. Dehority (2005) added that a pH value below 5.4 cause death to protozoa, and that too acidic or too basic rumen environment causes decrease in the effectiveness of microbial enzymes, therefore, stopping them from functioning altogether and consequently causing failure in the fermentation process, with optimum pH at 6.2 to 6.8 which matches that of roughage-fed animal.

Dry Matter Intake as Affected by Forage Extracts Defaunation

The dry matter intake (DMI) of goats during the defaunation period (day 1-4) and recovery period (day 5-7) showed significant differences, with those receiving the Desmodium (T_2) and Ipil-ipil (T_5) forage extracts having the highest and those with Robles (T_1) and SLS (control) having the lowest (Table 4). The lower DMI values in the defaunation period compared to that in the recovery period were also due to the 50% reduction in feed DM offered. It was also observed that goats receiving SLS treatment (control) became weak, and half of them suffered from scouring during the defaunation period, which could have contributed a lot to the great reduction in DMI. It can be gleaned from Table 4 that during the recovery period (day 4-7) DMI increased, showing the beneficial effects of defaunation on voluntary DM intake. Supporting the present study, Chandramoni *et al.* (2002) reported that higher feed intake levels were observed in rumen defaunated sheep.

The result also proved the beneficial effects of using forage extract in defaunating the rumen compared to the commonly used SLS detergent. Comparing the forage extracts of Ipil-ipil and Desmodium and SLS, having also higher percent reduction in protozoal numbers, DMI of the basal diet greatly

increased while that of SLS treatment had the lowest although it greatly reduced protozoal numbers. Although not measured in this study, Beauchemin *et al.* (2007) stated that addition of 2% quebracho tannin extract to the diet did not influence either DM or neutral detergent fiber (NDF) digestibility in cattle, but feeding very high levels of dietary saponins and/or tannins can reduce apparent digestibility (Poungchompu *et al.,* 2009).

Table 4. Dry matter intake of goats as affected by the administration of saponin-containing forage extracts.

Treatments	Day 1-4 (Defaunation)		Day 5-7 (Recovery)	
	DMI (kg)	DMI (% BW)	DMI (kg)	DMI (% BW)
SLS	0.058^{b}	0.538^{c}	0.202^{d}	1.864^{c}
Robles	0.096^{ab}	0.735^{bc}	0.207^{cd}	1.699^{c}
Desmodium	0.143^{a}	1.083^{a}	0.301^{ab}	2.464^{a}
Kakawate	0.109^{ab}	0.849^{abc}	0.244b^{cd}	1.941^{bc}
Centrosema	0.114^{ab}	0.877^{abc}	0.263a^{bc}	2.125^{abc}
Ipil-ipil	0.144^{a}	1.063^{ab}	0.305^{a}	2.395^{ab}
p – value	*0.009***	*0.001***	*0.001***	*0.039**

Means of similar letter-superscript within a column are not significantly different.
***Highly significant; *Significant*

The result also proved the beneficial effects of using forage extract in defaunating the rumen compared to the commonly used SLS detergent. Comparing the forage extracts of Ipil-ipil and Desmodium and SLS, having also higher percent reduction in protozoal numbers, DMI of the basal diet greatly increased while that of SLS treatment had the lowest although it greatly reduced protozoal numbers. Although not measured in this study, Beauchemin *et al.* (2007) stated that addition of 2% quebracho tannin extract to the diet did not influence either DM or neutral detergent fiber (NDF) digestibility in cattle, but feeding very high levels of dietary saponins and/or tannins can reduce apparent digestibility (Poungchompu *et al.,* 2009).

CONCLUSION AND RECOMMENDATION

Saponin-containing forages are effective defaunating agents comparable to sodium lauryl sulfate. Ipil-ipil (*Leucaena leucocephala*) and Robles (*Cassia siamea)* can effectively defaunate the rumen, but Ipil-ipil and Desmodium (*Desmodium heterophylum)* promote higher feed dry matter intakes than Kakawate *(Gliricidia sepium)* and Centro (*Centrosema pubescens)* leaf extracts.

While sodium lauryl sulfate is an effective defaunating agent, it can also cause a decrease in voluntary feed dry matter intake, hence, the use of saponin-containing forage extracts of Ipil-ipil or Desmodium are highly recommended. For practical application, such forages may be offered as feed supplement or part of the normal diet of goats.

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REFERENCES

ABAN, M.L 2012. Acceptability of foliage with varying pH levels from selected trees and shrubs in goats. Visayas State University, Visca, Baybay City, Leyte.

ABARGHUEI, M.J., Y. ROUZBEHAN and D. ALIPOUR. 2010A. The influence of the grape pomace on the ruminal parameters of sheep. *Livest Sci;* **132**: 73–79.

BA, N. B. and L. D. NGOAN. 2003. Evaluation of some unconventional trees/plants as ruminant feeds in Central Vietnam Hue University of Agriculture and Forestry. *Livestock Research for Rural Development.* **15**(6).

BABAYEMI, O.J., D. DEMEYER and V. FIEVEZ. 2004. Nutritive value and qualitative assessment of secondary compounds in seeds of eight tropical browse, shrub and pulse legumes. *Comm. Agric. Appl. Biol. Sci.*, **69**: 103-110.

BEAUCHEMIN, K.A., S.M. MCGINN, T.F. MARTINEZ and T.A. MCALLISTER. 2007. Use of condensed tannin extract from quebracho trees to reduce methane emissions from cattle. *J. Anim. Sci.* **85**: 1990-1996.

BUSQUET, M., S. CALSAMIGLIA FERRETA, and C. KAMEL. 2006. Plant extracts affect *in vitro* rumen microbial fermentation. *J Dairy Sci;* **89**: 761–771.

CHAUHAN, TR., M. UMANG, A. SAINI AND S.S. DAHIYA, V. B. DIXIT and D. LALL. 2004. Neem Leaves as a Defaunating Agent in the Diet of Buffalo Bull Calves Fed With Sorghum Straw on basal Diet. *Proceedings – Contributed papers World Buffalo Congress*, Makati City, Philippines. **2**(7):343 -345

CLARKE, R.T.J. 1977. Protozoa in the rumen ecosystem. In: *Microbial ecology of the gut* (T.T.J. Clarke and T. Bauchop, eds). New York: Academic Press, pp.251-275

DEHORITY, B.A. 2005. Effect of pH on viability of *Entodinium caudatum, Entodinium exiguum, Epidinium caudatum,* and *Ophryoscolex purkynjei in vitro. Journal of Eukaryotic Microbiology*. **52**:339-342.

DIAZ, A., M. AVENDANO and A. ESCOBAR. 1993. Evaluation of *Sapindus saponaria* as a defaunating agent and its effects on different ruminal digestion parameters. *Livestock Research for Rural Development.* **5**(2): 1-5

DUNDAR, Y. 2004. Effects of Defaunation and Urea on Glutathione and Malondialdehyde Levels in Blood and Ruminal Fluid of Ramlic Lambs. *Turk J Vet Anim Sci* **28**:265-269.

EUGENE, M., H. ARCHIMEDE, B. MICHALET-DOREAU, G. FONTY.2004. Effects of Defaunation on Microbial Activities in the Rumen of Rams Consuming a Mixed diet (fresh *Digitaria decumbens* grass and concentrate). **53**:187 – 2000

FRANCIS G., Z. KEREM, H.P.S. MAKKAR and K.. BECKER. 2002. The biological action of saponins in animal systems: Reviews. *Br. J. Nutr.,* **8**:587-605.

FRANZOLIN, R. and B.A. DEHORITY. 2010. The role of pH on the survival of rumen protozoa in steers. *Revista Brasileira de Zootecnia*. **39**(10):2262-2267

FULGUEIRA, C.L., S.L., AMIGOT, M. GAGGIOTTI, L.A. ROMERO, J.C. BASILICO. 2007. *Forage quality: Techniques for testing*. Fresh produce Global Science Books

GEBEYEHU, A. and Y. MEKASHA. 2013. Defaunation: Effects on Feed Intake, Digestion, Rumen Metabolism and Weight Gain. *Wudpecker Journal of Agricultural Research* **2**(5):134-141

KIRAN, D. and T. MUTSVANGWA. 2010. Effects of partial ruminal defaunation on urea-nitrogen recycling, nitrogen metabolism, and microbial nitrogen supply in growing lambs fed low or high dietary crude protein concentrations. *Journal of Animal Science* **88**(3):1034-47

LENG, R.A., S.H. BIRD, A. KLIEVE, B.S. CHOO, F.M. BALL, G. ASEFA, P. BRUMBY, V.D. MUDGAL, U.B. CHAUDHRY, S.U. HARYONO and N. HEDRATNO. 2011. The Potential For Tree Forage Supplements To Manipulate Rumen Protozoa To Enhance Protein To Energy Ratios In Ruminants Fed On Poor Quality Forages. *FAO* pp 1-15. Accessed last January 10, 2014. D:/cd3wddvd/NoExe/…/meister11.htm.

LILA, Z.A., N. MOHAMMED, S. KANDA, T. KAMADA and H. ITABASHI. 2003. Effect of sarsaponin on rumin with particular reference to methane production in vitro. *J Dairy Sci*; **86**: 3330–3336.

LOPEZ- CAMARENA, J., J.R. OROZCO – HERNANDEZ, J.H. MEDINA – VILLAREAL, J.J. URIBE – GOMEZ, H. VERDIN – SANCHEZ and V.O. FUENTES – HERNANDEZ. 2010. Evaluation Of Robenidine To Defaunate Pelibuey Lambs And The Production And Digestibility. *Journal of Animal and Veterinary Advances* **9**(1):44-46

MOSS, A.R., J.P. JOUANY and J. NEWBOLD. 2000. Methane production by ruminants: its contribution to global warming. *Ann. Zootech*. **49**: 231–253.

NEWBOLD, C.J., S.M.E. HASSAN, J. WANG, M.E. ORTEGA. and R.J. WALLACE 1997. Influence of foliage from African multipurpose trees on activity of rumen protozoa and bacteria. *Br. J. Nutr.*, **78**: 237-249

NGAMSAENG, A., M. WANAPAT and S. KHAMPA. 2006. Effects of Mangosteen Peel (*Garcinia mangostana*) Supplementation on Rumen Ecology, Microbial Protein Synthesis, Digestibility and Voluntary Feed Intake in Cattle. *Pakistan Journal of Nutrition* **5**(5): 445 – 452

OZDEMIR, M., M. CINAR, S. HALILOGLU and A. ERYAVUZ. 2006. Effects of Defaunation and Dietary Nitrogen Source on Sodium, Potassium, Iron and Zinc in the Rumen Fluid, Plasma and Wool of Lambs. *Turkish Journal of Veterinary and Animal Science* **30**:367-373

PATRA, A.K., D.N. KAMRA and N. AGARWAL. 2006. Effect of plant extracts on in vitro methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. In: *Anim. Feed Sci. Technol.*, **128**:276-291.

PELL, A.N., R.I. MACKIE, I. MUELLER-HARVEY, L.R. NDLOVU. 2001. Tannins: analysis and biological effects in ruminant feeds (special issue). *Anim Feed Sci Technol* **91**:112-113.

POUNGCHOMPU, O., M. WANAPAT, C. WACHIRAPAKORN, S. WANAPAT and A. CHERDTHONG. 2009. Manipulation of ruminal fermentation and methane production by dietary saponins from mangosteen peel and soapberry fruit. *J. Agricul. Sci*. **63**: 389-400.

RUIZ Y., A, MOUMEN, M.A.I, GARCIA M.E. and ALCAIDE. 2014. Ruminal fermentation and degradation patterns, protozoa population and urinary purine derivatives excretion in goats and wethers fed diets based on two-stage olive cake: Effect of PEG supply. *J Anim Sci*; **85**: 2023–2032.

SANTRA, A. and S.A. KARIM. 2000. Growth performance of faunated and defaunated Malpura weaner lambs. *Animal Feed Science and Technology*. **86**:251-260.

TEFEREDGNE, B., 2000. New perspectives on the use of tropical plants to improve ruminant nutrition. *Proc. Nutr. Soc.,* **59**: 209-214.

USHIDA K.,C., S. KAYOULI, D.E. SMET, and J. P. JOUANYS. 1990. Effect of Defaunation on protein and Fiber Digestion in Sheep fed on Ammonia – Treated Straw – Based Diets with or Without Maiza. Brtitish *Journal of Nutrition* **64**:765 -775

VALLESER, A. 2011. Effect of bypass Protein Supplementation on Defaunated Sheep (*Ovis aries L.)* Fed Napier Grass (*Pennisetum purpureum schumach*). Master's Thesis, Visayas State University, Visca, Baybay City, Leyte. pp. 15-25

WANAPAT, M. 2000. Rumen Manipulation to ncrease the Efficient Use of Local Feed and Productivity of Ruminants in the Tropics. *Asian-Australian. Journal of Animal Science. Vol.* 13

WILLIAMS, A and G. COLEMAN. 1991. *The Rumen Protozoa.* New York, NY, USA: Springer-Verlag.