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Effects of Daily and Interval Feeding of *Sapindus rarak* Saponins on Protozoa, Rumen Fermentation Parameters and Digestibility in Sheep

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ABSTRACT : Several researchers have demonstrated that the rumen microbial community rapidly adapts to saponins and proposed interval feeding to prevent this rapid adaptation. An *in vivo* experiment was carried out to examine the effect of daily versus application every third day (interval feeding) of *Sapindus rarak* saponins (SE) on rumen fermentation end products, protozoal counts and nutrient digestibility. Thirty sheep were allocated into 5 groups. Sheep were fed daily or every third day with two levels of SE (0.48 and 0.72 g/kg body mass). One group received no saponin and served as control. All sheep received the same diet, a mixture of elephant grass and wheat pollard (65:35 w/w). Independent of the feeding regime and the level of inclusion, the addition of SE decreased protozoal counts and rumen ammonia concentrations ($p<0.01$). Microbial N supply and N retention were not affected by the high feeding regime. Daily feeding negatively influenced rumen xylanase and cellulase activity, but only when the high level of saponins was fed. However, these negative effects on rumen cell wall degradation were not reflected in decreasing total tract digestibility of the organic matter or the plant cell walls. Our results show that rumen microorganisms do not rapidly adapt to *S. rarak* saponins. (**Key Words :** Digestibility, Interval Feeding, Rumen, Saponin, *Sapindus rarak*)

INTRODUCTION

There are several reports on the utilization of saponin containing plants or saponin extracts on ruminants and monogastric animals aiming to improve animal production (see reviews of Cheeke, 2000; Francis et al., 2002; Wallace et al., 2002; Takahashi et al., 2005). In ruminants, saponins may suppress protozoa, resulting in a lower predation of bacteria by protozoa. This often results in a larger bacterial population and a less intraruminal protein turnover in the rumen leading to an increase in bacterial N flow to the duodenum (Cheeke, 1996; Hess et al., 2004). However, several reports have shown that the defaunating effect of saponins in the rumen is not always persistent. Saponin-containing *Enterolobium cyclocarpum* leaves depressed protozoal counts only during the first few days of feeding (Leng et al., 1992, Ivan et al., 2004). *Sesbania sesban* saponins defaunated the rumen microbial community *in vitro,* but these effects were not persistent *in vivo* and protozoal counts increased markedly after several days (Newbold et al., 1997; Odenyo et al., 1997; Teferedegne et al., 1999; Teferedegne, 2000). Based on this only result, Newbold et al. (1997) suggested to feed saponins intermittently to slow down the adaptation of rumen microorganisms to the saponins.

Sapindus rarak (Sapindaceae) is a tall tree which originated in South East Asia and is now widely distributed in Asia. The fruit pericaps have a foaming property in water and traditionally is used as natural soap for washing. Saponins have been reported to be present in these fruits (Wina et al., 2003), which have opened to include these fruits in the animal diet as an additive to manipulate rumen fermentation (Wina et al., 2006). Addition of *S. rarak* saponins to *in vitro* decreased significantly protozoal counts, RNA concentrations of *Ruminococci* and *Chytridiomycetes* (fungi) and xylanase activity in the rumen (Wina et al., 2005). Only one report from Thalib et al. (1996) showed that feeding *Sapindus rarak* saponin extract every third day kept the protozoal counts low even after 3 weeks. This trial was conducted to investigate the response of ruminal protozoa and rumen fermentation and nutrient digestibility in sheep, when a *Sapindus rarak* saponin extract was fed

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Table 1. Composition of dietary components (g/kg dry matter)

	Organic matter	Nitrogen	Neutral detergent fibre	Gross energy (MJ/kg DM)
Elephant grass	895	13.7	737	15.9
Wheat pollard	952	25.1	322	16.5

either daily or in interval of three days.

MATERIALS AND METHODS

Preparations of saponin extract from *Sapindus rarak* fruit pericarps (SE)

Sapindus rarak fruits (from Central Java, Indonesia) were dried in the oven at 60°C, The seeds were removed and the fruit pericarps were ground to powder. The powder was soaked overnight in methanol (1:4 w/v). After settling of the particles, the extract was decanted and the extraction was repeated. From the combined extracts, the methanol was evaporated in a rotary evaporator and the remaining water was removed by freeze drying. The extract was then stored in air-tight containers to avoid rehydration.

Digestibility trial

Thirty male sheep (thin-tailed local Indonesian breed) were kept in the individual wooden slatted cage, about 0.75 m above the ground. Initial body mass of the sheep was 16.5±1.8 kg. The diet consisted of a mixture of elephant grass (*Pennisetum purpureum*) and wheat pollard in the ratio of 65:35 w/w at a level of 4% of body mass per day. The proximate composition of elephant grass and wheat pollard is presented in Table 1. The diet contained Crude protein, Neutral detergent fibre and gross energy content of the diet was 110 g/kg, 592 g/kg and 16.1 MJ/kg, respectively. Calcium in the form of limestone and salt were added at the level of 12 g/kg and 3.3 g/kg.

The sheep were grouped into 5 treatments; One group received no saponins and served as control (C). The saponin extract was fed at a high (0.72 g/kg body mass) and a low level (0.48 g/kg body mass). The saponins were fed either daily or in interval of three days. The *Sapindus rarak* extract (SE) was mixed with wheat pollard and was offered in the morning before grass feeding to ensure complete intake of the saponins offered. The unconsumed grass was recorded daily before the morning feeding and subsample of feed refusal was collected and analysed. The sheep were fed for 27 days and the collection of urine and faeces was carried out from day 18 to day 26. Rumen liquor was taken from each sheep on day 25 and day 27.

Sample collection

Faeces were collected every day for a period of 9 days. A bulk of faeces was weighed every morning before the morning meal and a representative subsample (about 10%) was taken, oven dried at 60°C and dry matter was determined. Daily samples were then combined for further analysis.

Urine was collected every morning in a bucket containing 100 ml of 2.6 N sulphuric acid solution. Volume was made up to 2 litres with water and an aliquot was taken and stored at -20°C. Daily samples from each animal were then pooled and were analysed for nitrogen and purine derivative concentration.

Rumen liquor (50 ml) was taken from each sheep in the morning before feeding using a plastic stomach tube and filtered through two layers of cheesecloth. For Short Chain Fatty Acid (SCFA) analysis, an aliquot of 1.5 ml was centrifuged at 14,000 g for 10 min. The supernatant (0.9 ml) was mixed with 0.1 ml of formic acid containing 10 ml/L methyl valeric acid as an internal standard and kept at 4°C overnight. The next day, the mixture was centrifuged at 14,000 g for 10 min and the clear supernatant was transferred into a glass vial and kept at 4°C. The pellet obtained after the first step of centrifugation was kept at -40°C for the analysis of cellulase and xylanase activity. An aliquot (0.3 ml) of the filtered rumen liquor was collected for protozoal counts. One ml of filtered rumen liquor was collected for the determination of the ammonia concentration by the Conway method (Conway and Byrne, 1933).

Chemical analysis

Faeces and feed refusal were analysed for dry matter (DM) and organic matter (OM) according to Association of Official Analytical Chemists (1984). Nitrogen content of faeces, urine and feed refusal was determined by Kjeldahl digestion and measured by autoanalyser (Technicon method). The method of Van Soest et al. (1991) without addition of amylase was used to determine the neutral detergent fibre (NDF) content of faeces and feed refusal. Gross energy was measured using a bomb calorimeter.

Short chain fatty acids were analysed on a gas chromatography (GC-14A, Shimadzu Corporation, Japan, Tokyo) fitted with a flame ionization detector. Separation was carried out using a stainless steel column packed with GP 107, SP 1,000/L % H_3PO_4 on Chromosorb WAW (100/120 mesh) (Holtershinken et al., 1997).

Xylanase and CMCase activities were measured as the amount of sugar released from oat spelt xylan and Carboxymethylcellulose (CMC), respectively according to the method of Groleau and Forsberg (1983).

Protozoa were fixed in a solution containing 40 ml/L formaldehyde, 13.5 mmol/L NaCl and 0.6 mg/ml methyl green and counted in an improved Neubauer Chamber.

Concentrations of allantoin, uric acid, xanthine and hypoxanthine in the urine were determined by the method described by International Atomic Energy Agency (1997).

Table 2. Protozoal count, end products of fermentation and enzyme activity in the ruminal fluid of sheep fed different dosages (low or high) of SE with daily or interval feeding

Parameter	Control	Daily feeding		Interval feeding		SED	Contrasts		
		Low	High	Low	High		C-SE	FR	Dose
Protozoa (10^5/ml)	16.2	3.9	3.5	5.1	5.9	2.4	***	ns	ns
Ammonia-N (mg/L)	136	111	99	128	114	0.01	**	#	#
SCFA (mmol/ml)	54.9	56.0	50.6	51.7	56.0	3.4	ns	ns	ns
Acetate (mol %)	71.0	70.6	71.0	71.4	71.7	0.74	ns	ns	ns
Propionate (mol %)	17.6	19.9	20.0	18.6	18.9	0.98	*	ns	ns
n-Butyrate (mol %)	8.4	7.0	6.3	7.2	6.9	0.50	***	ns	ns
CMCase (mg/ml h^{-1})	4.1	3.2	2.7	3.9	3.1	0.43	*	*	*
Xylanase (mg/ml h^{-1})	32.7	26.7	12.8	26.8	21.2	4.7	**	**	**

High = 0.72 g SE/kg body mass, Low = 0.48 g SE/kg body mass.
C = Control; SE = *S. rarak* extract; FR = Daily versus interval feeding.
ns = Non significant; # = Approaching significance ($p<0.1$); * $p<0.05$; ** $p<0.01$; *** $p<0.001$.
SED = Standard error for difference.

Calculation of microbial protein supply

Microbial protein supply and efficiency of microbial protein supply were calculated by formula constructed by Chen and Gomes (1992) based on the total purine derivatives excretion in the urine.

Excretion of purine derivatives (Y) :

Y (mmol/d) = allantoin+uric acid
+(xanthine+hypoxanthine)

Absorption of microbial purines (X):

$$Y \text{ (mmol/d)} = 0.84X+(0.150\ BM^{0.75}\ e^{-0.25X})$$

$$\text{Microbial N supply (g N/day)} = \frac{X \text{ (mmol/d)}\times 70}{0.83\times 0.116\times 1{,}000}$$

$$= 0.727\ X$$

The digestibility of microbial purines is assumed to be 0.83
The N content of purines is 70 mg N/mmol
The ratio of purine N : total N in mixed rumen microbes is taken as 11.6:100
Efficiency of microbial protein supply (EMPS)

$$\text{EMPS (g N/kg DOMR)} = \frac{\text{Microbial N supply}}{\text{DOMR}}$$

DOMR (digestible organic matter apparently fermented in the rumen) = 0.65 of the DOMI (digestible organic matter intake)

Statistical analysis

Statistical analysis was done using the GLM procedure in SAS package version 8 (2000). Data were analysed using one-way variance analysis. Contrast analysis was conducted to detect significant treatment effects, between the control and SE addition; between daily and interval feeding as well as between low (0.48 g/kg BM) and high (0.72 g/kg BM) level of SE.

RESULTS

Rumen parameters

Table 2 shows that the addition of SE into the diet significantly decreased protozoal counts ($p<0.001$). The feeding regime (daily versus interval feeding) did not significantly affect the protozoal counts but tended to decrease protozoal counts at both saponin levels. The dose of saponins did not affect the protozoal counts.

S. rarak saponins decreased the ammonia concentration in the rumen ($p<0.01$). Daily feeding of saponins especially at high level tended to decrease the ammonia concentration ($p<0.1$). SCFA concentration in the rumen was not significantly affected either by the level of saponins or by the feeding regime. SCFA composition was altered by saponin feeding. The *S. rarak* extract enhanced molar proportion of propionate ($p<0.05$) but depressed butyrate ($p<0.001$) without affecting acetate. Cellulase and xylanase activities, in contrast, were affected by both, the feeding regime and the saponin dose. Higher dose of saponins and daily feeding significantly decreased the activity of both enzymes.

Intake, digestibility and nitrogen balance

Table 3 shows that SE did not significantly depress any intake or digestibility of various dietary fractions. However, there are trends for lower digestibility of organic matter and plant cell wall when the high dosage of saponins was applied. Although daily feeding of high SE lowered the intake and the digestibility, the difference to the other treatments was not significant. The nitrogen intake only tended to be lower ($p<0.1$) with daily feeding of SE than

Table 3. Intake and digestilibility of diet on sheep fed different dosages (low and high) of SE with daily or interval feeding

Parameter	Control	Daily feeding		Interval feeding		SED
		Low	High	Low	High	
Intake (g/kg BM 0.75)						
Organic matter	59.2	58.0	49.3	60.3	61.9	5.47
Neutral detergent fibre	39.5	38.8	32.0	40.5	42.3	4.60
Nitrogen	1.21	1.19	1.06	1.23	1.20	0.06
Digestibility (g/kg)						
Organic matter	621	609	591	612	587	24.8
Neutral detergent fibre	512	509	463	524	500	28.9
Nitrogen	713	702	707	713	665	24.3

High = 0.72 g SE/kg body mass; Low = 0.48 g SE/kg body mass.
Contrast analysis is not presented as none of these parameters is significantly different.

Table 4. Nitrogen balance in sheep fed different dosages (low and high) of SE on daily or interval feeding

Parameter	Control	Daily feeding		Interval feeding		SED
		Low	High	Low	High	
Nitrogen balance (g/d)						
N-intake	9.60	9.30	9.19	9.57	10.45	0.66
Faecal N	2.79	2.79	2.70	2.78	3.53	0.37
Urinary N	1.70	1.65	1.50	1.43	1.71	0.28
N-retention	5.11	4.88	5.00	5.37	5.21	0.6
Microbial N supply (g/d)	1.12	1.33	1.17	1.13	1.58	0.31
EMPS (gN/kg DOMR)	5.97	7.46	7.33	6.20	7.76	1.73

High = 0.72 g SE/kg body mass; Low = 0.48 g SE/kg body mass.
EMPS = Efficiency of microbial protein synthesis.

with interval feeding.

SE did not significantly affect nitrogen balance in sheep. Relative to the N intake, the nitrogen retention is rather high, but no significant effect of the treatments could be observed. The nitrogen excretion in faeces and urine was also not affected by the dosage of SE or the feeding regime (Table 4). Microbial N supply increased by 19% and 41% and the efficiency of microbial protein synthesis (EMPS) also improved by 25% and 30% when the sheep were fed low SE daily and high SE every third day, respectively. However, none of these differences reached a significant level.

DISCUSSION

The effect on protozoa

The rumen liquor was taken on days 25 and 27 of the experiment and the protozoal counts in the rumen liquor from SE-fed sheep were still significant lower than those in the control sheep independent of the feeding regime (Table 2). The lack of a dose dependent defaunation response indicates that maximum defaunation effect was already reached with the lower dose of saponins. The present result suggested that protozoa community does not rapidly adapt to the *S. rarak* saponins. Another feeding trial with increasing level of SE feeding supported this result (Wina et al., 2006). The present result is in contrast to several reports that showed a recovery of protozoal counts after several days of feeding. Newbold et al. (1997) observed that protozoal numbers fell by 60% after 4 days of feeding saponin-containing *Sesbania sesban* leaves to sheep, but the protozoal population recovered after 14 days. A similar adaptation of rumen protozoa to saponins from *Enterolobium cyclocarpum* was shown by Ivan et al. (2004). Partial defaunation was achieved for 11 days, but the protozoal population had recovered completely after 14 days. All these authors assumed that there was a quick adaptation of mixed microbial population in the rumen which was one factor contributing to the loss of anti protozoal activity of saponins. Teferedegne et al. (1999) and Odenyo et al. (1997) suggested that the loss of the anti protozoal activity of the saponins could be due to the degradation of saponins in the rumen by deglycolysation. This can be a fast process once the bacterial population has adapted. Within the genus Sapindus results are mostly obtained from *S. saponaria*. Results from these plants are variable reaching from no defaunation effect (Hess et al., 2003; Abreu et al., 2004) to considerable partial defaunation (Hess et al., 2004). The diet and the previous exposure of the experimental animals to saponin-containing forages may influence the effect of saponins. Very little work has been done with *S. rarak* saponins. To our knowledge, only Thalib et al. (1996) used the same saponin extract placed in a capsule and it could partially defaunate the rumen of sheep over 21 days. Together with our results, it indicates that *S. rarak* saponins differ considerably in their defaunating activity from those of *S. saponaria*. The content of saponins in *S. rarak* extract (7.5%, Hamburger et al., 1992) was

lower than that in *S. saponaria* (12%, Abreu et al., 2004).

The chemical structure of saponins from *Sapindus rarak* fruit pericarps is almost similar to those reported in *Sapindus saponaria* fruit pericarps. Both species have monodesmoside triterpenoid saponins that possess hederagenin as the agylcone (Hamburger et al., 1992; Lemos et al., 1992, 1994). Differences occur mainly in the sugar composition and arrangement. In *Sapindus saponaria*, glucose is directly attached to hederagenin and rhamnose and arabinose are linked to the glucose (Lemos et al., 1992, 1994). In *Sapindus rarak,* arabinose is attached to hederagenin and rhamnose and xylose as sugar residues attached to arabinose (Hamburger et al., 1992). Since saponins loose their defaunating activity upon deglycolysation by rumen microorganisms (Teferedegne et al., 1999), the type of sugars and their chemical linkages may influence their degradation by microbes and, therefore, their defaunating activity. On the other hand, sugar composition and linkage is also directly related to the activity of the saponins (Oleszek et al., 1990; Woldenmichael and Wink, 2001; Jung et al., 2004). Chwalek et al. (2004) demonstrated that the (1→4) linkage in hederagenin diglycosides had a higher haemolytic activity than (1→6) linkage. Moreover, β linkage configuration in hederagenin diglycosides also showed a higher activity than α linkage configuration. Jung et al. (2004) showed that rhamnose contributed more cytotoxic activity than glucose in oleanane digylcosides isolated from *Akebia quinata*. The observed differences in the activity of saponins of closely related species might be partly due to differences in their chemical composition.

Effect on rumen fermentation

Addition of SE to the diet decreased ammonia concentration in the rumen (Table 2) but as observed for other parameters, this did not occur in a dose dependent manner. Other researchers also observed a decreased ammonia concentration through administration of saponins. Yucca saponin extract decreased ruminal ammonia concentration in cows (Husain and Cheeke, 1995), heifers or steers (Hristov et al., 1999; Lila et al., 2005) and sheep (Śliwiński et al., 2002). The lower ammonia concentrations are due to a lower intraruminal nitrogen turnover as a consequence of defaunation. Defaunation and refaunation experiments have clearly shown the influence of protozoa on ruminal ammonia concentration (Ivan et al., 2000). However, other factors like a direct influence of saponins on proteolytic activity (Wallace et al., 1994) may affect ammonia concentrations, but no effect of *S. rarak* saponins was detected on protein degradation or proteolytic activity in the rumen (Muetzel et al., 2005).

Total SCFA production was not affected by treatments, but defaunation increased the proportion of propionate, while acetate and butyrate, the major end products of protozoal metabolism were decreased. Several researchers observed changes in molar proportions of SCFA towards higher propionate production through defaunation mainly in *in vitro* (Lu et al., 1987; Hess et al., 2003, Lila et al., 2003). As observed for protozoal counts and ammonia production, neither the feeding regime nor the saponins level affected SCFA concentration or the molar proportions of individual SCFA to a significant extent.

In contrast to the above results, both CMCase and xylanase enzyme activities were depressed by SE (Table 2). For these parameters, the effect was dose dependent and daily feeding had a higher effect than the interval feeding. Apparently, the *S. rarak* saponins negatively affected fibrolytic microorganisms. This is in line with the results of Wang et al. (2000) who showed a reduction in growth of various rumen microorganisms in response to Yucca saponins. In previous *in vitro* study, we found that *S. rarak* saponins negatively affected *Ruminococcus albus*, *R. flavefaciens* and *Chytridiomycetes* but not *Fibrobacter* sp. (Wina et al., 2005). However, protozoa also exerted several fibrolytic enzymes (William and Withers, 1991) so defaunation itself may have contributed to the lower fibrolytic enzyme activity.

Although ruminal plant cell wall degradation was decreased by SE, the total tract NDF digestibility was not affected either by the dose of saponins or the feeding regime (Table 3). Kreuzer and Kirchgessner (1988) found that defaunation decreased the ruminal digestion of organic matter, fibre and protein, but due to an increase in hindgut fermentation the total tract digestibility was less affected. Lu and Jorgensen (1987) also observed an increase in fibre degradability in the hindgut when cellulose degradability in the rumen was reduced by the addition of alfalfa saponin. The lack of an effect on total tract digestibility in our experiments might, therefore, be explained by the compensation of the rumen effects in the hindgut.

Effect on intake, nitrogen balance and microbial N supply

Sheep readily consumed wheat pollard containing *S. rarak* extract and the bitterness of *S. rarak* extract did not distract the sheep. Intake was not affected by all treatments. The slightly reduced intake of the organic matter and the NDF at the highest absolute saponin dose could be a reflection of the lower ruminal cell wall degradation since rate of ruminal cell wall degradation and intake of roughages are linked (van Soest, 1994). However, in contrast to ruminal cell wall degradation, the effect on intake was not significant. This is in agreement with other authors where saponins did not affect the intake of the animals (Hristov et al., 1999; Hess et al., 2004; Śliwiński et al., 2004; Lila et al., 2005).

In the present experiment, there was an increase in the efficiency of the microbial protein synthesis with SE feeding but this increase was not significant (Table 4). Defaunation can lead to an increased microbial biomass supply to the duodenum since the efficiency of microbial protein synthesis is increased by the lack of protozoal predation on bacteria. Makkar and Becker (1996) found that the efficiency of *in vitro* microbial protein synthesis (EMPS) linearly increased by the addition of quillaja saponins (0.4 to 1.2 mg/ml) to a hay substrate. Our previous *in vitro* result also showed that an increase in microbial biomass occurred due to *S. rarak* saponin (Wina et al., 2006). The lack of a significant increase in microbial N supply in our experiment may partly be due to the high variation among animals. This result is, however, in agreement with the results reported by Hess et al. (2004), Śliwiński et al. (2002) and Hristov et al. (1999) where no effect on microbial protein supply upon the administration of saponins was observed. Another explanation of the lack of an effect on microbial N supply to the animal might be that SE only partially defaunates the rumen and the composition of the protozoal population affects their impact on the microbial N flow. Ivan et al. (2000) observed that the presence *Polyplastron multivesiculatum*, *Epidinium ecaudatum, Eudiplodinium maggii* and *Entodinium ecaudatum* decreased the flow of non-ammonia N, bacterial N and total amino acids from the rumen to the intestinal tract, while *Isotricha intestinalis* and *Dasytricha ruminantium* have very little effect. Since protozoal genera were not determined separately in our experiment such an effect cannot be excluded.

No effect of SE on N excretion was observed and consequently, the nitrogen retention was not affected as well. The N-retention was comparatively high as the N excretion was low due to the low N-content of the diet. Hess et al. (2004) also found that *Sapindus saponaria* fruit did not increase N retention as it increased the nitrogen excretion through faeces. Inconsistent results were reported by Eugène et al. (2002) and Eugène et al. (2004), which showed that defaunation may or may not influence nitrogen retention depending on the diet.

CONCLUSION

S. rarak saponin extract, in spite of its bitter taste, was readily consumed when mixed with a more palatable feed ingredient. *S. rarak* saponins are effective to partially defaunate the rumen without loosing their activity within 27 days. Interval feeding as suggested by other researchers in order to prevent the rapid adaptation of the microbial community to saponins is not required. Although *S. rarak* saponins decreased ruminal ammonia concentrations, no effects on N excretion or retention was observed. The negative effects of *S. rarak* saponins on the ruminal fibrolytic enzyme did not influence total tract digestibility of the plant cell wall.

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REFERENCES

Abreu, A., J. E. Carulla, C. E. Lascano, T. E. Diaz, M. Kreuzer and H. D. Hess. 2004. Effects of *Sapindus saponaria* fruits on ruminal fermentation and duodenal nitrogen flow of sheep fed a tropical grass diet with and without legume. J. Anim. Sci. 82:1392-1400.

Association of Official Analytical Chemists, 1984. Official Methods of Analysis, 14th edition. AOAC, Arlington, VA.

Cheeke, P. R. 1996. Biological effects on feed and forage saponins and their impacts on animal production. In Saponins Used in Food and Agriculture (Ed. G. R. Waller and Y. Yamasaki), Plenum Press, New York, pp. 377-385.

Cheeke, P. R. 2000. Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition. In Proceedings of the American Society of Animal Science, Indiapolis 10p. 1999. See: http://www.asas.org/JAS/symposia/proceeding/0909.pdf

Chen, X. B. and M. J. Gomes. 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives - an overview of the technical details. Occasional Publications IFRU, Rowett Research Institute, Aberdeen, UK. (Ed. M. Chwalek, K. Plé and L. Voutquenne-Nazabadioko) 2004. Synthesis and hemolytic activity of some hederagenin diglyosides. Chem. Pharm. Bull. 52:965-971.

Conway, E. J. and A. Byrne. 1933. An absorption apparatus for the determination of certain volatile substances. I. The micro-determination of ammonia. Biochem. J. 27:419-429.

Eugène, M., H. Archimede, B. Michalet-Doreau and G. Fonty. 2004. Effects of defaunation on digestion of mixed diet (fresh *Digitaria decumbens* grass and concentrate) at four protein to energy ratios. Anim. Res. 53:111-125.

Eugène, M., H. Archimede, J. L. Weisbecker, F. Pommier, F. Nipeau and D. Sauvant. 2002. Effects of defauntion on digestion of fresh *Digitaria decumbens* grass and growth of lambs. In: Effects de la défaunation de ruminants sur les performances de production, en function de la ration ingérée. Etude des variations de la protéosynthése et de la cellulolyse microbienne ruminale. (M. Eugène). PhD thesis. Institut National Agronomique, Paris-Grignon, France.

Francis, G., Z. Keem, H. P. S. Makkar and K. Becker. 2002. The biological action of saponins in animal systems : a review. Br. J. Nutr. 88:587-605.

Groleau, D. and C. W. Forsberg. 1983. Partial characterization of the extracellular carboxymethylcellulase activity produced by

the rumen bacterium *Bacteroides succinogenes*. Can. J. Microbiol. 29:504-517.

Hamburger, M., I. Slacanin, K. Hostettmann, W. Dyatmiko and Sutarjadi. 1992. Acetylated saponins in molluscicidal activity from *Sapindus rarak*: Unambiguous structure determination by proton nuclear magnetic resonance and quantitative analysis. Phytochem. Anal. 3:231-237.

Hess, H. D., R. A. Beuret, M. Lötscher, I. K. Hindrichsen, A. Machmüller, J. E. Carulla, C. E. Lascano and M. Kreuzer. 2004. Ruminal fermentation, methanogenesis and nitrogen utilization of sheep receiving tropical grass hay-concentrate diets offered with *Sapindus saponaria* fruits and *Cratylia argentea* foliage. Anim. Sci. 79:177-189.

Hess, H. D., L. M. Monsalve, C. E. Lascano, J. E. Carulla, T. E. Diaz and M. Kreuzer. 2003. Supplementation of a tropical grass diet with forage legumes and *Sapindus saponaria* fruits: effects on *in vitro* ruminal nitrogen turnover and methanogenesis. Aust. J. Agric. Res. 54:703-713.

Holtershinken, M., U. Plitt, F. C.Tammen, P. Hoffmann and H. Scholz. 1997. Influence of mouldy grass on fermentation and thiamine metabolism in bovine rumen fluid (*in vitro*) Deut. Tier. Wochenschrift 104:17-22.

Hristov, A. N., A. McAllister, F. H. Van Herk, K. J. Cheng, C. J. Newbold and P. R. Cheeke. 1999. Effect of *Yucca schidigera* on ruminal fermentation and nutrient digestion in heifers. J. Anim. Sci. 77:2554-2563.

Hussain, I. and P. R. Cheeke. 1995. Effect of dietary *Yucca schidigera* extract on rumen and blood profiles of steers fed concentrate or roughage-based diets. Anim. Feed Sci. Tech. 51:231-242.

International Atomic Energy Agency. 1997. Estimation of rumen microbial protein from purine in urine. IAEA Technical Document (TECDOC) 945.

Ivan, M., K. M. Koenig, B. Teferedegne, C. J. Newbold, T. Entz, L. M. Rode and M. Ibrahim. 2004. Effect of the dietary *Enterolobium cyclocarpum* foliage on the population dynamics of rumen ciliate protozoa in sheep. Small Rum. Res. 52:81-91.

Ivan, M., L. Neill and T. Entz. 2000. Ruminal fermentation and duodenal flow following progressive inoculations of fauna-free wethers with major individual species of ciliate protozoa or total fauna. J. Anim. Sci. 78:750-759.

Jung, H. J., C. O. Lee, K. T. Lee, J. W. Choi and H. J. Park. 2004. Structure-activity relationship of oleannane disaccharides isolated from *Akebia quinata* versus cytotoxicity against cancer cells and NO inhibition. Biol. Pharm. Bull. 27:744-747.

Kreuzer, M. and M. Kirchgessner. 1988. Effect of rumen protozoa on metabolism and retention in ruminants with special reference to diet characteristics. In: The Roles of Protozoa and Fungi in Ruminant Digestion (Ed. J. V. Nolan, R. A. Leng and D. I. Demeyer), Penambul Books Armidale, NSW, pp. 189-198.

Lemos, T. L. G., A. L. Mendes, M. P. Sousa and R. Braz-Filho. 1992. New saponin from *Sapindus saponaria*. Fitoterapia 93:515-517.

Lemos, T. L. G., M. P. Sousa, A. L. Mendes and R. Braz-Filho. 1994. Saponin from *Sapindus saponaria*. Fitoterapia 95:557-558.

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. Hendratno. 1992. The potential for tree forage supplements to manipulate rumen protozoa to enhance protein to energy ratios in ruminants fed on poor quality forages. In Legume trees and other fodder trees as protein sources for livestock. FAO Animal Production and Health paper 102 (Ed. A. Speedy and P. L. Pugliese), FAO, Rome, Italy, pp. 177-191.

Lila, Z. A., N. Mohammed, S. Kanda, T. Kamada and H. Itabashi. 2003. Effect of sarsaponin on ruminal fermentation with particular reference to methane production *in vitro*. J. Dairy Sci. 86:3330-3336.

Lila, Z. A., N. Mohammed, S. Kanda, M. Kurihara and H. Itabashi. 2005. Sarsaponin effects on ruminal fermentation and microbes, methane production, digestibility and blood metabolites in steers. Asian-Aust. J. Anim. Sci. 18:1746-1751.

Lu, C. D. and N. A. Jorgensen. 1987. Alfalfa saponins affect site and extent of nutrient digestion in ruminants. J. Nutr. 117:919-927.

Lu, C. D., L. S. Tsai, D. M. Schaefer and N. A. Jorgensen. 1987. Alteration of fermentation in continuous culture of mixed rumen bacteria by isolated alfalfa saponins. J. Dairy Sci. 79:799-805.

Makkar, H. P. S. and K. Becker. 1996. Effect of Quillaja saponins on *in vitro* rumen fermentation. In: Saponins Used in Food Agriculture (Ed. G. R. Waller and K. Yamasaki), Plenum Press, New York and London, pp. 387-394.

Muetzel, S., R. Akpagloh and K. Becker. 2005. Sapindus rarak saponins did not affect protein degradation *in vitro*. Proc. Soc. Nutr. Physiol. 14:73.

Newbold, C. J., S. M. El Hassan, J. M. 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.

Odenyo, A. A., P. O. Osuji and O. Karanfil. 1997. Effect of multipurpose tree (MPT) supplements on ruminal ciliate protozoa. Anim. Feed Sci. Technol. 67:169-180.

Oleszek, W., K. R. Price, I. J. Colquhoun, M. Jurzysta, M. Ploszynski and G. R. Fenwick. 1990. Isolation and identification of alfalfa (*Medicago sativa* L.) root saponins: their activity in relation to a fungal bioassay. J. Agric. Food Chem. 38:1810-1817.

Preston, T. R. 1995. Tropical Animal Feeding. A manual for research workers. FAO Animal Production and Health Paper 126, FAO, Rome, Italy.

Statistical Analysis Systems. 2000. SAS Procedures Guide release 8.0 SAS institute Inc., Cary, NC.

Śliwiński, B. J., M. Kreuzer, H. R. Wettstein and A. Machmüller. 2002. Rumen fermentation and nitrogen balance of lambs fed diets containing plant extracts rich in tannins and saponins and associated emissions of nitrogen and methane. Arch. Anim. Nutr. 56:379-392.

Śliwiński, B. J., M. Kreuzer, F. Sutter, A. Machmüller and H. R. Wettstein. 2004. Performance, body nitrogen conversion and nitrogen emission from manure of dairy cows fed diets supplemented with different plant extracts. J. Anim. Feed Sci. 13:73-91.

Takahashi, J., B. Mwenya, B. Santoso, C. Sar, K. Umetsu, T. Kishimoto, K. Nishizaki, K. Kimura and O. Hamamoto. 2005. Mitigation of methane emission and energy recycling in

animal agricultural systems. Asian-Aust. J. Anim. Sci. 18: 1199-1208.

Teferedegne, B., F. McIntosh, P. O. Osuji, A. Odenyo, R. J. Wallace and C. J. Newbold. 1999. Influence of foliage from different accessions of the subtropical leguminous tree, *Sesbania sesban* on ruminal protozoa in Ethiopian and Scottish sheep. Anim. Feed Sci. Technol. 78:11-20.

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

Thalib, A., Y. Widiawati, H. Hamid, D. Suherman and M. Sabrani. 1996. The effects of saponin from *Sapindus rarak* fruit on rumen microbes and performance of sheep. J. Ilmu Ternak Vet. 2:17-20 (Indonesia).

Van Soest, P. J. 1994. Nutritional Ecology of the ruminant, 2nd ed. Cornell University Press, USA.

Van Soest, P. J., J. B. Roberston and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and non starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Wallace, R. J., L. Arthaud and C. J. Newbold. 1994. Influence of *Yucca shidigera* extract on ruminal ammonia concentrations and ruminal microorganisms. Appl. Environ. Microbiol. 60:1762-1767.

Wallace, R. J., N. R. McEwan, F. M. McIntosh, B. Teferedegne and C. J. Newbold. 2002. Natural products as manipulators of rumen fermentation. Asian-Aust. J. Anim. Sci. 15:1458-1468.

Wang, Y., T. A. McAllister, L. J. Yanke, Z. J. Xu, P. R. Cheeke and K. J. Cheng. 2000. *In vitro* effects of steroidal saponins from *Yucca schidigera* extract on rumen microbes. J. Appl. Microbiol. 88:887-896.

Williams, A. G. and S. E. Withers. 1991. Effect of ciliate protozoa on the activity of polysaccharide-degrading enzymes and fibre breakdown in the rumen ecosystem. J. Appl. Bacteriol. 70:144-155.

Wina, E., S. Muetzel, E. Hoffman, H. P. S. Makkar and K. Becker. 2003. The effect of secondary compounds in forages on the rumen microorganisms quantified by 16S and 18S rRNA. Proceedings of International Symposium held in Vienna, Austria, 2003. Application of Gene-based Technology for improving animal production and health in developing countries. pp. 397-410.

Wina, E., S. Muetzel, E. Hoffman, H. P. S. Makkar and K. Becker. 2005. Saponins containing methanol extract of *Sapindus rarak* affect microbial fermentation, microbial activity and microbial community structure *in vitro*. Anim. Feed Sci. Technol. 121:159-174.

Wina, E., S. Muetzel and K. Becker. 2006. The dynamics of major fibrolytic microbes and enzyme activity in the rumen in response to short and long term feeding of *Sapindus rarak* saponins. J. Appl. Microbiol. 100:114-122.

Woldenmichael, G. M. and M. Wink. 2001. Identification and biological activities of triterpenoid saponins from *Chenoposium quinoa*. J. Agric. Food Chem. 49:2327-2332.