RUMEN FERMENTATION CHARACTERISTICS AND METHANOGENESIS IN SHEEP FED SILAGE-BASED DIET SUPPLEMENTED WITH *YUCCA SCHIDIGERA* OR *YUCCA SCHIDIGERA* COMBINED WITH NISIN

Budi Santoso[1]

ABSTRACT

Three rumen-fistulated wethers were used in a 3 × 3 Latin square design to determine the effect of supplementing *Yucca schidigera* with or without nisin on rumen fermentation characteristics and methane production in sheep fed a basal diet containing timothy silage and concentrate (85:15) on DM basis. Three treatments diet were basal diet (control); basal diet plus 240 ppm of *Yucca schidigera* (yucca); basal diet plus *Yucca schidigera* and 6 mg/kg $BW^{0.75}$ of nisin (yucca+nisin). Rumen pH was maintained between 6.56 and 6.62 and was found higher ($P<0.01$) in sheep receiving yucca and nisin supplemented diet compared to sheep receiving control diet. Concentration of NH_3-N reduced ($P<0.05$) in sheep receiving supplemented diets compared to control diet. There was a reduction ($P<0.01$) in CH_4 production (l/kg $BW^{0.75}$ and g/kg DM intake) in yucca or yucca plus nisin supplemented diets compared to control diet. These results indicated that supplementation of *Yucca schidigera* alone or combined with nisin could maintain normal fermentation in the rumen and reduce CH_4 emission by sheep.

(Key words : *Yucca schidigera*, Nisin, Rumen fermentation, Methane).

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[1]Fakultas Peternakan Perikanan dan Ilmu Kelautan Universitas Negeri Papua, Manokwari.

KARAKTERISTIK FERMENTASI RUMEN DAN METANOGENESIS PADA DOMBA YANG DIBERI PAKAN DASAR SILASE YANG DISUPLEMENTASI DENGAN *YUCCA SCHIDIGERA* ATAU KOMBINASI *YUCCA SCHIDIGERA* DENGAN NISIN

INTISARI

Tiga ekor domba berfistula pada bagian rumen digunakan dalam rancangan bujur sangkar Latin 3 × 3 untuk mengetahui pengaruh suplementasi *Yucca schidigera* dengan atau tanpa nisin terhadap karakteristik fermentasi rumen dan produksi metan pada domba yang diberi pakan basal terdiri dari silase timothy dan konsentrat. Tiga perlakuan pakan yaitu pakan basal (kontrol); pakan basal dengan suplementasi *Yucca schidigera* 240 ppm (yucca); pakan basal dengan suplementasi *Yucca schidigera* dan nisin 6 mg/kg $BB^{0,75}$ (yucca+nisin). Nilai pH dalam rumen dipertahankan antara 6,56 sampai dengan 6,62 dan lebih tinggi ($P<0,01$) pada domba yang diberi pakan dengan suplementasi yucca dan nisin dibandingkan pakan kontrol. Konsentrasi NH_3-N menurun ($P<0,05$) pada domba yang mendapat pakan bersuplementasi dibandingkan pakan kontrol. Terdapat penurunan ($P<0,01$) produksi metan (l/kg $BB^{0,75}$ dan g/kg konsumsi bahan kering) pada perlakuan yucca atau kombinasi yucca dengan nisin dibandingkan kontrol. Hasil penelitian ini mengindikasikan bahwa suplementasi *Yucca schidigera* atau dikombinasi dengan nisin dapat mempertahankan fermentasi rumen secara normal dan menurunkan produksi metan pada domba.

(Kata kunci : *Yucca schidigera*, Nisin, Fermentasi rumen, Metan).

Introduction

Methane (CH_4) is produced as a result of anaerobic fermentation of the soluble and structural carbohydrates by methanogens in the rumen of ruminant animals, which is released into the environment by eructation. The CH_4 emission from ruminant animals range from 2 to 12 %, meanwhile an average CH_4 production from sheep has been estimated as 7.22 % of the gross energy intake or 22.15 g/day (Pelchen and Peters, 1998). This means that CH_4 production from ruminant not only represents a substantial loss in efficiency of animal production, but also contributes significantly to global warming as the greenhouse gas.

Recently, there is an increasing interest in research activities to evaluate the potential of natural products as feed additives instead of chemical compounds as manipulators of rumen fermentation to improve animal performance. In the previously reported by Wallace *et al.* (2002), consumers and health authorities in developed countries have dictated that the use of chemical feed additives including antibiotics and ionophores should be phased out and, where possible only natural products should be used in animal production. Natural products as feed additives, however, should be harmless to animals, no toxic residue present in their bodies or animal products, and safe to the environment.

Extract of the *Yucca schidigera* plant contains a glycofraction that has NH_3-binding capabilities and a saponin fraction that has antiprotozoal and bacterial effects (Wallace *et al.*, 1994). Since 20 % of rumen methanogens are parasitic on the surface of protozoan body (Stumm *et al.*, 1982), CH_4 generation is clarified to decrease by defaunation. In the previous *in vitro* studies have shown that an extract of *Yucca schidigera* plant was effective to suppress CH_4 production (Wang *et al.*, 1998; Takahashi *et al.*, 2000). Whilst, nisin is a low molecular weight antimicrobial protein produced by certain strains of *Lactococcus lactis* subp. *Lactis*. Nisin has a mode of action similar to ionophores, which shows antibacterial activity mainly against lactic acid bacteria and Gram-positive bacteria including

methanogens and is widely used in the food industry as safe and natural preservative (Delves-Brougton *et al.*, 1996). Recent work has indicated that nisin reduced *in vitro* CH_4 production (Callaway *et al.*, 1997). Moreover, *in vivo* study (Santoso *et al.*, 2003a) found that addition of 120 ppm of *Yucca schidigera* reduced rumen methanogenesis in sheep.

The objective of the present study was to assess the effect of supplementing *Yucca schidigera* with or without nisin on rumen fermentation characteristics and methane production in sheep fed a diet containing timothy silage and concentrate.

Materials and Methods

Design, animals and diets

Three Cheviot wethers (54.5 ± 5.4 kg BW) fitted with ruminal fistula were kept in the ventilated head cages equipped container for urine and faeces collections, and assigned to three treatments in a 3 × 3 Latin square design. Sheep were fed in two equal meals (08.00 and 16.00 h) at the feeding level of 55 g dry matter (DM)/kg$BW^{0.75}$ per day with a basal diet comprised timothy silage and concentrate (85:15) on DM basis (Table 1). The treatments were as follows; basal diet (control); basal diet supplemented with 240 ppm of *Yucca schidigera* per day (yucca); basal diet supplemented with 240 ppm of *Yucca schidigera* and 6 mg/kg $BW^{0.75}$ of nisin per day (yucca+nisin). Both *Yucca schidigera* and nisin were top-dressed on the basal diet. *Yucca schidigera* is available in a powder form (94.4 % DM) under commercial name DK Sarsaponin 35 (Desert King International), and the amount recommended by the manufacture's is 60 ppm of complete ration. The nisin powder used was the commercial preparation (Sigma Chemical Co., USA), containing 4 mg nisin/g. The sheep always had ad libitum access to fresh water and a mineral block. Each experimental period consisted of 8 days for adaptation followed by 3 days for rumen fluid collection and gas measurement. Eight days of dietary adjustment was allowed for adaptation of rumen microbial to the treatments (Grubb and Dehority, 1975).

Rumen fluid collection and analyses

Rumen fluid samples were taken from the middle part of rumen by using a 100 ml syringe immediately before morning feeding and 1, 2, 3, 4, 5 and 6 h after feeding. The pH value was measured immediately with a digital pH meter (Toa, HM-21P, Japan). Thereafter, the samples were kept frozen at –20 °C for later determination of NH_3-N and VFA concentrations as previously described (Santoso *et al.*, 2003b).

CH_4 production measurement

Methane production was measured by an open circuit respiratory system using ventilated hood attached to each metabolism cage for 2 consecutive days. During each measurement, air was continuously sampled from ventilated hood and analysed for CH_4 concentration with infrared analyser (Horiba VIA 300, Japan). The CH_4 gas volume was converted to mass value using the conversion factor 0.716 g/l.

Statistical analysis

Analysis of variance was carried out using the GLM procedure of SAS (version 6.12; SAS Institute Inc., Cary, NC USA) as a Latin square design. Data on rumen pH, NH_3-N and VFA collected each sampling time were analyzed by a split-plot analysis of variance. In case of significant difference in treatment effect, the Duncan's multiple range test was performed to separate treatment means.

Table 1. Chemical composition (%) of silage, concentrate and mixed diet on DM basis

	Timothy silage	Concentrate[1]	Silage-based diet[2]
Dry matter	29.1	87.4	37.8
Organic matter	90.8	93.2	91.2
Crude protein	13.4	24.1	14.9
NDF	55.8	12.9	49.4
ADF	37.0	7.1	32.5

[1]Contained heat treated corn, maize, soybean and rye (52 %), soybean oil (34 %) corn gluten feed and alfalfa mill (8 %), molasses, $CaCO_3$ powder, Dicalcium phosphate, salt, malt, yeast, lactic acid bacteria, bacillus, Streptococcus, vitamins and minerals (vitamin A, 34350 IU/kg; vitamin D3, 6870 IU/kg; vitamin E, 46 mg/kg; Zn, 229 mg/kg; Mn, 126 mg/kg; Fe, 69 mg/kg; Cu, 33 mg/kg; I, 2.6 mg/kg; Co, 0.8 mg/kg; Se, 0.46 mg/kg) (6 %).
[2]85 % timothy silage and 15 % concentrate on DM basis.

Results and Discussion

Rumen fermentation characteristics

Table 2 summarizes the pH value, concentrations of NH_3-N and VFA in rumen fluid of the experimental sheep. The concentrations of ammonia-N and total VFA, and ratio of acetate and propionate in rumen fluid were affected by sampling time ($P<0.05$). Rumen pH value was higher ($P<0.01$) in sheep receiving yucca and nisin supplemented diet compared to control diet. This higher pH value may reflect a decrease in total VFA concentration in sheep fed yucca and nisin supplemented diet that presumably could be due to lower activity of holotrich protozoa. Coleman (1979) demonstrated holotrich protozoa are able to rapidly ferment soluble sugars and produce VFA and lactic acid. These results, however, are in agreement with previous finding of Wilson *et al.* (1998) that *Yucca schidigera* tended to increase pH in the rumen of cows when added to diets with low or high soluble protein. In the present study, rumen pH was maintained between 6.50 and 6.62, which were in optimum pH ranges (6.7 ± 0.5) to maintain normal cellulolytic (Van Soest, 1994).

Ammonia-N concentration in the rumen is a function of the balance between production, absorption and incorporation into microbial matter. The sheep had lower ($P<0.05$) rumen NH_3-N concentration when supplemented with yucca alone or yucca combined with nisin versus control diet. Decreased of NH_3-N in sheep fed supplemented diets compared to control diet might be due to lower deamination of amino acids coming from the degradation of dietary and microbial protein in the rumen by ciliate protozoa. In agreement with this result, Takahashi *et al.* (2000); Wang *et al.* (1998) noted that supplemental yucca extract in *in vitro* ruminal culture fermentations decreased NH_3 concentration. In contrast, Wu *et. al.* (1994) reported steroid saponins of *Yucca schidigera* plant did not significantly affect concentration of NH_3-N in ruminal fluid of dairy cows. So far, there are several statements concerning the optimum rumen NH_3-N level for fibre digestion. Mehrez *et al.* (1977) suggested the rumen ammonia concentration of 193 mg N/l is minimum value for maximal rate of roughage fermentation, whereas Abdulrazak *et al.* (1997) concluded that rumen ammonia concentration of 50 – 80 mg/l could be sufficient for fibre digestion. In the present study, however, concentration of NH_3-N in all treatment diets was above the minimum value for fibre digestion.

Table 2. Rumen fermentation characteristics of sheep fed silage-based diet supplemented with *Yucca schidigera* with or without nisin[1]

Variables	Treatment			SE	Significance	
	Control	Yucca	Yucca+nisin		Treatment	Time
pH	6.50[b]	6.56[ab]	6.62[a]	0.057	**	NS
NH_3-N (mg/l)	248.2[a]	227.1[b]	221.5[b]	10.92	*	*
Total VFA (mM)	57.3	55.9	54.9	2.81	NS	*
Molar proportion of VFA (mol/100 mol)						
Acetate (A)	67.6	66.7	66.9	0.70	NS	NS
Propionate (P)	16.5	16.7	16.2	0.38	NS	NS
Butyrate	9.1[b]	10.4[a]	10.5[a]	0.38	**	NS
Valerate	3.6	3.4	3.5	0.37	NS	NS
Iso-acids[2]	2.1	2.3	2.3	0.21	NS	NS
A:P	4.2	4.1	4.2	0.12	NS	*

Means in the same row with different superscripts are significantly different.
[1]Each value is a mean for seven determinations per animal
[2]Including iso-butyrate and iso-valerate
SE : standard error of the mean
NS : not significant, * $P<0.05$, ** $P<0.01$.

Total VFA concentration ranged from 54 to 57 mM in the present study and was in normal total VFA concentration (France and Siddons, 1993). Total VFA concentration of ruminal fluid was slightly decreased in sheep receiving diets supplemented with yucca alone or combined with nisin compared to control diet. However, lower total VFA concentration in supplemented diets than those of control diet may be related to the reduced activity of rumen microorganism. A significantly higher ($P<0.01$) proportion of butyrate was observed in sheep fed supplemented diets than that of control diet, which agreed with *in vivo* study by Wu *et al.* (1994).

Methane production

Sheep fed yucca or yucca combined nisin supplemented diets had lower ($P<0.01$) CH_4 production expressed as l/kg $BW^{0.75}$ compared to control diet (Table 3). Lower ($P<0.01$) CH_4 production expressed as g/kg DM intake was also observed in sheep receiving yucca and nisin supplemented diet versus control diet or yucca supplemented diet. The CH_4 production expressed as g/kg DM intake for yucca supplemented diet or yucca and nisin supplemented diet was reduced by 8.0 % and 13.5 %, respectively compared to control diet. Previous work has shown that CH_4 production (g/kg DM intake) reduced about 5.6 % by addition of 120 ppm of *Yucca schidigera* (Santoso *et al.*, 2003). These results indicate CH_4 production from sheep reduced with increasing concentration of yucca in the diet. Average CH_4 production (g/kg DM intake) in the present study was, however, slightly higher than the value of 22.2 g reported by Santoso *et al.* (2003a). The reduction in CH_4 production after supplementation was relatively greater in *Yucca schidigera* combined with nisin than that of *Yucca schidigera* alone. It has been reported that the cell of methanogens such as *Methanobrevibacter ruminantium* and *Methanosarcina barkeri* are gram positive and lack peptidoglycan to protect the cell membrane (Moss, 1993). Callaway *et al.* (1997) revealed that nisin has a mode of action similar to inophores that are highly lipophilic substance, which can move ions across membrane. Moreover, Montville and Chen (1998), nisin has a higher affinity for lipid monolayers of anionic lipids, which are

Table 3. Methane production of sheep fed silage-based diet supplemented with *Yucca schidigera* with or without nisin

Variables	Treatment			SE	Significance
	Control	Yucca	Yucca + nisin		
Methane production					
l/kg $BW^{0.75}$	1.88[a]	1.71[b]	1.65[b]	0.033	**
g/d	26.5	24.1	22.9	0.71	NS
g/kg DM intake	25.2[a]	23.2[ab]	21.8[b]	0.85	**

Means in the same row with different superscripts are significantly different.
SE : standard error of the mean
NS : not significant. ** $P<0.01$.

predominant in Gram-positive bacteria. Whilst, lower CH_4 production in *Yucca schidigera* supplemented diet could be explained by a decreased of protozoa numbers by saponins of yucca, consequently reducing population of methanogens in the rumen. As reported by Wallace *et al.* (1994); Wang *et al.* (1998), saponins of *Yucca schidigera* have antimicrobial properties that particularly in suppressing ciliate protozoa. Klita *et al.* (1996) reported that presence of cholesterol in eukaryotic (including protozoal) cell membranes, but not in prokaryotic bacterial cell is a possible selective susceptibility of ruminal ciliate protozoa to saponins. Moreover, Hegarty (1999) suggested that reduced methanogenesis induced by the absence of protozoa is a secondary response to direct rumen changes including loss of methanogens associated with protozoa. Consequently, reduced carbohydrate fermentation and protozoal H_2 production depressed and rumen O_2 potential changed in the absence of protozoa. Previous studies have shown that about 9 – 25 % of methanogens are parasitic on the surface of protozoan body (Stumm *et al.*, 1982).

Conclusion

Addition of 240 ppm of *Yucca schidigera* alone or combined with 6 mg/kg $BW^{0.75}$ of nisin to silage-based diet could maintain normal rumen fermentation and reduce CH_4 emission by sheep. Thus, natural products such as extract *Yucca schidigera* plant or nisin have great potential to be used as manipulators of rumen fermentation.

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