Nitrogen Metabolim in Sheep Fed Hay-Based Diet Supplemented with *Yucca schidigera* with or without Nisin

(Metabolisme Nitrogen pada Domba yang Diberi Pakan Hay dan Suplementasi *Yucca schidigera*)

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Abstrak

Empat ekor domba yang berfistula pada bagian rumen digunakan pada rancangan Bujur sangkar latin 3 × 3. Penelitian ini bertujuan untuk mengetahui pengaruh supplementasi *Yucca schidigera* dengan atau tanpa nisin terhadap penggunaan nitrogen, konsentrasi amonia dalam rumen, konsentrasi nitrogen urea dalam plasma, suplai nitrogen mikroba pada domba yang diberi pakan basal hay rumput timothy dan konsentrat. Tiga perlakuan pakan yaitu pakan basal (kontrol), pakan basal + *Yucca schidigera* 240 ppm (yucca), pakan basal + *Yucca schidigera* 240 ppm + nisin 6 mg/kg $BW^{0.75}$ (yucca+nisin). Ekskresi nitrogen dalam urin domba yang diberi perlakuan yucca+nisin lebih rendah ($P<0.05$) dibandingkan perlakuan kontrol. Perlakuan yucca+nisin meningkatkan keseimbangan nitrogen sebesar 8 % dibandingkan kontrol. Konsentrasi amonia dalam rumen pada perlakuan yucca dan yucca+nisin lebih rendah ($P<0.05$) dari pada perlakuan kontrol, dan konsentrasi tersebut masih berada diatas konsentrasi minimal untuk pertumbuhan mikroba dalam rumen. Konsentrasi nitrogen urea dalam plasma tidak dipengaruhi oleh perlakuan dan bervariasi antara 20.8 – 22.0 mg/dl. Suplai nitrogen mikroba pada perlakuan yucca dan yucca+nisin lebih tinggi ($P<0.05$) dibandingkan perlakuan kontrol. Berdasarkan hasil penelitian ini dapat disimpulkan bahwa suplementasi yucca atau kombinasi yucca dan nisin dapat menurunkan konsentrasi amonia dalam rumen, sehingga menurunkan ekskresi nitrogen pada urin dimana hal ini berimplikasi positif dalam mengurangi pencemaran nitrogen di lingkungan

Kata Kunci : *Yucca schidigera*, Nisin, Penggunaan N, N Mikroba

Introduction

Protein consumed by ruminants are partly broken down to peptides, amino acids and finally to ammonia (NH_3) by the bacteria and protozoa in the rumen. Increasing dietary protein or nitrogen intake generally is accompanied by increasing NH_3 concentration. Excess quantities of NH_3 in the rumen is absorbed into the blood stream, converted to urea in the liver and subsequently excreted in the urine, which is related to environmental pollution (Tamminga, 1992).

The extract of *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). Accordingly, a small amount of *Yucca schidigera* should bind to a significant amount of NH_3 when NH_3 concentration is high after feeding. Therefore, *Yucca schidigera* slowly releases NH_3 for use during synthesis of microbial protein as concentration of NH_3 decreased.

Previous study (Santoso *et al.*, 2003a) has indicated that feeding 120 ppm of *Yucca schidigera* to sheep fed silage based diet was effective to reduce rumen NH_3 concentration resulting in low nitrogen losses in urine, therefore improved efficiency of nitrogen utilization by up to 50 %. Meanwhile, nisin is bacteriocin produced by certain strain of *Lactococcus lactis* subsp *lactis* and is widely used in the food industry as a safe and natural preservative (Delves Broughton *et al.*, 1996). Callaway *et al.* (1997) reported that nisin inhibited growth of *Clostridium amoniphylum*, which is obligate amino-acid fermenting bacteria.

However, increasing of concentration of yucca and nisin or its combination, in order to increase nitrogen utilization, may have a different response with hay-based diet.

The objective of this experiment was to determine the manipulating effect of *Yucca schidigera* with or without nisin on nitrogen utilization, rumen NH_3-N and plasma urea-N (PUN) concentrations in sheep given hay-based diet.

Research Methods

Animals and diets

Four ruminally fistulated Cheviot wethers with an initial body weight (BW) of 54.5 ± 5.4 kg were kept in the metabolic cages equipped with containers for urine and feces collection and allocated in a 3 × 3 Latin square design. The animals were fed twice daily (08:00 h and 16:00 h) at maintenance level of energy (55 g dry matter (DM)/kg $BW^{0.75}$ per day) with a basal diet comprised timothy hay and concentrate (60:40), on DM basis (Table 1).

The commercial concentrate contained, on a DM basis, 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 %).

Three experimental diets were basal diet (control), basal diet + 240 ppm of *Yucca schidigera* (Yucca), and basal diet + 240 ppm of *Yucca schidigera* and 6 mg/kg $BW^{0.75}$ of nisin (Yucca + nisin).

Yucca and nisin were supplemented by sprinkling onto the feed. *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 used was purchased from Sigma Chemical Co. (St.Louis, MO, USA) containing 4 mg nisin/g.

Water and a mineral lick containing Fe 1232; Cu 150; Co 25; Zn 500; I 50; Se 15 and Na 382 mg/kg were freely available. Each 14 day experimental period consisted of 8 days of dietary adjustment, 5 days of nitrogen balance study, and 1 day of rumen fluid and blood collections.

Nitrogen balance study

Total feces and urine were collected daily for a 5-day period. Feed refusals, if any were removed daily before morning feeding. Representative samples of the hay and concentrate were taken before experiment, oven dried at 60 °C for 72 h and ground to pass a 1 mm screen. Feces output was weighed daily, pooled per treatment on a 5-day basis.

Table 1. Chemical composition (g/kg) of silage, concentrate and mixed diet

	Timothy hay	Concentrate	Hay-based diet[1]
Dry matter	917	874	900
Organic matter	947	932	941
Crude protein	85	241	147
NDF	746	129	499
ADF	464	71	307

60 % timothy hay and 40 % concentrate, on DM basis.

About 10% of fecal samples were taken and stored at –20 °C for subsequent analyses. The DM, organic matter (OM) and N of feed and fecal samples were deter-mined by AOAC (1990) procedures, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analysed according to procedures by Van Soest *et al.* (1991). Both the NDF and ADF contents were measured on an ash-free basis. Urine was collected into buckets containing 100 ml of 10 % sulphuric acid to reduce pH below 3 and prevent bacterial destruction of purine derivatives. An amount of 5 % urine was collected and frozen at –20 °C until analysis of total N. Additional sub-sample was diluted by five-fold with distilled water and was stored at –20 °C for analyses of purine derivatives (Chen and Gomes, 1995).

Rumen fluid and blood collections

On the last day of each experimental period, rumen fluid samples were taken from the abdominal sac of rumen by using a hand syringe immediately before morning feeding and 1, 2, 3, 4, 5 and 6 h after feeding. The samples were stored at –20 °C for later analysis of NH_3-N concentration as previously described by Santoso et al. (1993b). Blood samples were collected from the jugular vein 3 h after morning feeding into heparinized vacuums tube. Plasma was separated by centrifugation of whole blood for 15 min at 1,500 × g, and then frozen at –20°C for later PUN determination (commercial kit Urea N B).

Estimation of microbial nitrogen supply and statistical analysis

Microbial N flow to duodenum was estimated from urinary purine derivatives excretion according to Chen and Gomes (1995). Data were subjected to analysis of variance using the general linear models procedure of SAS (Statistical Analysis Systems, version 6.12; SAS Institute Ins., Cary, NC USA). The significant treatment differences were compared using Duncan's multiple range test.

Results and Discussion

Nitrogen Balance

Data on N metabolism of sheep given hay-based diet with supplementation of *Yucca schidigera* or *Yucca schidigera* combined with nisin is summarized in Table 2. Sheep fed yucca+nisin supplemented diet had lower ($P<0.05$) urinary N output, which resulted in a higher retained N compared to sheep fed control diet. Loss of N in urine originates from various sources such as rumen loss, the replacement of metabolic losses in the gut, and the incorporation of dietary N into microbial nucleic acids. Urinary N is mainly composed of urea (65 – 90%), and the remaining consists of considerable amounts of allantoin, hippuric acid, creatinine, creatine and uric acid (Tamminga *et al.*, 1995). Decreased in urinary N excretion as a proportion of N intake obtained in this study was associated with decreasing rumen NH_3 concentration (Table 2 and Table 3), which is consistent with the finding reported by Lewis (1957). Moreover, Nolan (1993) pointed out that excess ammonia produced for microbial protein synthesis is absorbed directly across the rumen wall, and also from the large intestine, into the blood stream. Although most of the blood ammonium is converted to urea in the liver, all of the urea formed is not excreted in the urine, because some is being recycled in the body. However, reducing in urinary N excretion in yucca+nisin supplemented sheep may have a positive impact to abate N pollution to the environment either as ammonia in the air or as nitrate in soil and ground water.

Table 2. Nitrogen balance and nitrogen partition of sheep fed hay-based diet supplemented with *Yucca schidigera* with or without nisin

	Treatment			SE	Significance
	Control	Yucca	Yucca+nisin		
Nitrogen balance (g kg $BW^{-0.75}$ per day)					
N intake	1.29	1.28	1.28	0.01	NS
Fecal N	0.30	0.33	0.32	0.01	NS
N Digested	0.98	0.95	0.95	0.02	NS
Urinary N	0.63^{a}	0.59^{ab}	0.57^{b}	0.01	*
Retained N	0.35	0.37	0.38	0.02	NS
Nitrogen partition (% of N intake)					
Fecal N	23.7	25.7	25.2	0.73	NS
Urinary N	48.8^{a}	46.4^{ab}	44.9^{b}	0.68	*
Total loss N	72.5	72.1	70.1	1.11	NS
Retained N	27.5	27.9	29.9	1.11	NS

Means in the same row with different superscripts are significantly different.
SE: standard error of the mean
NS = not significant. * $P<0.05$.

Table 3. Rumen ammonia-N and plasma urea-N concentrations, and microbial N supply of sheep fed hay-based diet supplemented with *Yucca schidigera* with or without nisin

	Treatment			SE	Significance
	Control	Yucca	Yucca+nisin		
Rumen NH_3-N (mg/l)	260.6^{a}	244.8^{b}	223.3^{c}	0.17	**
Plasma urea-N (mg/dl)	22.0	21.0	20.8	3.23	NS
Microbial N supply (g/d)	10.7^{b}	12.4^{a}	11.4^{ab}	0.41	*

Means in the same row with different superscripts are significantly different.
SE: standard error of the mean
NS = not significant; *P<0.05; ** P<0.01.

Retained N in sheep receiving yucca + nisin supplemented diet was increased by about 8 % compared to sheep receiving the control diet. This is further supported by compensatory decrease of urinary N output and increase of microbial N supply. Total N losses as a proportion of N intake in supplemented diets was slightly lower than control diet (71.1 vs. 72.5 %).

Rumen ammonia-N and plasma urea nitrogen

Table 3 shows NH_3-N concentration was lower ($P<0.05$) in sheep fed on supplemented diets than those fed on control diet. Decreased of NH_3-N concentration in sheep given supplemented diets than control diet could be attributed to decreased deamination of amino acids coming from the degradation of dietary and microbial protein in the rumen by ciliate protozoa. In agreement with this result, Wallace *et al.* (1994); Santoso *et al.* (1993a) noted supplementation of yucca extract decreased NH_3 concentration in *in vitro* ruminal culture fermentations and *in vivo* experiment. Another possible reason was glycofraction of yucca that has the binding capability could slow down the rate of NH_3 release, and then this condition might be useful in increasing efficiency of NH_3 fixation into microbial protein.

Ammonia-N concentration in the rumen is a function of the balance between production, absorption and incorporation into microbial matter (Nolan, 1993). There is a lot of disagreement in the literature concerning the optimum rumen NH_3-N level for microbial growth. Satter and Slyter (1974) noted that concentration of 50 mg NH_3-N/l is optimal level to support maximum growth rates of rumen bacteria. On the other hand, Okorie *et al.* (1977) recommended value of 70 mg N/l for maximum microbial protein synthesis. In the present study, however, concentration of NH_3-N in all treatment diets was more than the minimum values for microbial growth.

There was no significant difference ($P>0.05$) observed in PUN concentration among the three treatments. Sheep fed on supplemented diets had a slightly lower concentration of PUN compared to control diet. A similar response on PUN concentration between un-supplemented diet and supplemented diet was consistent to previous finding of Wilson *et al.* (1998) who reported cows fed *Yucca schidigera* extract had PUN concentration that were similar to those of control cows fed diets without yucca extract. In the present study, the tendency for a decrease in PUN concentration by *Yucca schidigera* with or without nisin-supplemented diets was consistent to rumen NH_3-N concentration. Lewis (1957) stated that blood urea level seems to reflect the overall changes in ammonia production in the rumen. Concentration of PUN obtained in the present study ranged from 20.8 to 22.0 mg/dl was similar to concentrations suggested by Rowlands *et al.* (1974). However, in this study blood samples were taken only 3 h after feeding that the results may not offer complete information on PUN.

Microbial N supply

The excretion of purine derivatives is a well accepted for estimating microbial protein synthesis in the rumen (Chen and Gomes, 1995). Microbial N supply was higher ($P<0.05$) in sheep fed on yucca-supplemented diet than those fed on control diet (Table 3).The reason for high microbial N supply in yucca-supplemented sheep could be due inhibiting effect of saponins of *Yucca schidigera* on ciliate protozoa number. In consequence, increasing the proliferation of bacteria participating in rumen fermentation and then resulting in higher the amount of microbes that passes to the small intestine. Ushida et al. (1991) reported that protozoa to ingest and digest bacteria as their main protein source. The ingestion and digestion capacities by single protozoa are estimated to be 10^2 – 10^4 bacteria per hour. Furthermore, defaunation could improve efficiency of bacterial protein synthesis expressed as bacterial N per unit of fermented organic matter by 40 – 125 % (Jouany and Ushida, 1999). Lower microbial N supply values in yucca and nisin supplemented sheep than those of yucca supplemented sheep could be explained by depressing contribution of bacteria protein that passed to duodenum as a result of nisin inhibition in the rumen, which agreed with *in vitro* study by Jalč and Lauková (2002).

Conclusion

Results indicate that extract of *Yucca schidigera* plant and nisin have the potential to reduce NH_3-N concentration in the rumen and to increase microbial N supply to duodenum. The combination between yucca and nisin had the lowest urinary N excretion may have a positive impact to abate N pollution to the environment. Sheep fed on supplemented

diets had better in N utilization than sheep fed on control diet.

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