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RESEARCH ARTICLE

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Research on *Yucca schidigera* extract feeding on the rumen ecology, protozoal populations and serum chemistries of sheep

Abstract In a completely randomized block design experiment, 16 ruminally cannulated male sheep with body weights of (40 ± 2.1) kg were fed twice daily (8:00 and 16:00) with concentrate and forage (50:50 on dry matter (DM) basis). Dietary treatments were supplemented with intraruminal doses of powdered *Yucca schidigera* extract (YSE) at the levels of 0 (control), 100, 200 and 300 mg·kg^{-1}. On days of 15, 16 and 17 after feeding, ruminal content was sampled at 0, 2, 4, 6 and 8 h after dosing (8:00), and blood samples were collected at the end of experiment (the days 18 and 19 after feeding). Results showed that the treatment groups' acidity was not affected ($P = 0.13$) by YSE. Comparing to the control, the ruminal propionate concentration was increased by YSE addition in a dose-dependent manner by up to 29.8% ($P < 0.05$), and the acetic concentration was decreased by up to 17.5% ($P < 0.05$). The ruminal ammonia concentration 2 hours after feeding was higher ($P < 0.05$) in sheep fed without YSE (increased by 17.57 mg·100 mL^{-1}) than those fed with YSE at 200 mg·kg^{-1} (6.77 mg·100 mL^{-1} increase in NH_3) and at 300 mg·kg^{-1} (6.50 mg·100 mL^{-1} increase in NH_3). Protozoal populations in the rumen were lower ($P < 0.05$) with the YSE feeding dose at 300 mg·kg^{-1} than the control. The serum chemistries were not different among treatments ($P > 0.05$) and were within the normal physiological ranges for sheep 19 days after feeding. The study indicated that 200 mg·kg^{-1} and 300 mg·kg^{-1} YSE groups had particular suppressing effects on ruminal ammonia concentration, ammonia-N concentrations and protozoal populations. The effect of YSE on ruminal fermentation could be attributed to the selective inhibitory effect on rumen microbial species. High level (300 mg·kg^{-1}) YSE as feed additives resulted no negative impact on sheep in our tests.

Keywords *Yucca schidigera* extract, rumen ecology, protozoal populations, serum chemistries, sheep

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1 Introduction

In recent years, there has been considerable interest in identifying natural products and the optimal conditions of using them to manipulate ruminal fermentation and to improve livestock production. *Yucca shidigera* grows in the south-western deserts of the United States and in the Baha California region of Mexico. *Yucca schidigera* extract (YSE) has been used as a safe food material for both humans and livestock. Adding YSE to animal feeds could enhance animal performance, bind with ammonia, improve the ruminal environment and inhibit urease activity. All of these benefits have been well documented in many different research papers. Researches have shown some stimulatory effects of YSE added to diets on chickens and rabbits (Al-Bar et al., 1993), laying quails (Ayasan et al., 2005), goats (Aregheore, 2005) and awassi lambs (Kaya et al., 2006). A number of studies *in vitro* and *in vivo* showed that YSE can decrease ruminal ammonia levels

and increase propionate concentration (Hristov et al., 1999; Santoso et al., 2004; Santoso et al., 2006; Singer et al., 2008), inhibit methane production (Lila et al., 2003; Pen et al., 2006), and alter the rumen microflora *in vivo* (Killeen et al., 1998). Because of its strong ammonia binding capacity, YSE can be used to decrease the ammonia concentration of barns, increase laying rate and egg weight of laying quails (Kaya et al., 2003), increase milk yields (Singer et al., 2008), enhance average daily gain of chicks and reduce the faecal aroma of canine and feline animals (Giffard et al., 2001). Average daily gain can be improved by fed high-grain diets supplemented with YSE in finishing steers (Lovett et al., 2006). It is believed that YSE and its bioactive properties, together with further studies on ruminal fermentation, will draw more and more attention in the livestock industry.

The objectives of this study were (1) to find the effects of YSE on protozoal populations and some ruminal fermentation characteristics i.e., pH, concentrations of ammonia-N and volatile fatty acids (VFA), and then determine the optimal dosage of YSE. This is the basis for the application of YSE on ruminal production; (2) to investigate the safety of the highest level (300 mg·kg^{-1}) of YSE as a feed additive for sheep through serum traits.

2 Materials and methods

The *Yucca shidigera* extract (YSE) used in our study was in a solid powder form (92% DM) and contained 30% saponins (70% inactive carriers) supplied by Alltech Co., Ltd. (Beijing, China).

2.1 Animals and feeding

Sixteen ruminally cannulated male sheep with body weights (BW) of (40 ± 2.1) kg were selected in this experiment. All the sheep had good appetite and were equally arranged in 4 pens according to a single factorial randomized block design. They were weighted at the beginning of the experiment and then at each 19-day interval (14 days of adaptation to treatment and 5 days for sample collection). The sheep were fed twice daily, at 8 am and 4 pm, at the maintenance level of energy with a basal diet of 50% commercial concentrate and 50% *Leymus chinensis* as forage (DM basis; Table 1). *Leymus chinensis* is a perennial grass widely distributed in northern China and it is important for both the economy and in ecology. YES was added in feeds at four different dosages of 0 (control), 100, 200 and 300 mg·kg^{-1}.

2.2 Laboratory analyses

Feed samples were dried at 60°C for 72 h in a forced-air oven, ground through a 1 mm mesh sieve and later analyzed for DM (934.01) and Kjeldahl N (988.05) according to the methods of AOAC (1990). NDF and ADF were analyzed according to the methods of van Soest et al. (1991). Soluble crude protein (SCP) was assayed using the borate–phosphate buffer method of Licitra et al. (1996) with the minor modification of not using the sodium azide solution.

2.3 Sampling and analyses

2.3.1 Ruminal content samples

On days 15, 16 and 17 in the experiment, samples of the whole ruminal liquid were collected right before the feeding (0 h) and 2, 4, 6 and 8 h after the morning feeding at 8 am. Approximately 30 mL liquid from the ventral sac was combined with another 30 mL liquid from the reticulum. The mixture was filtered through four layers of cheesecloth (Hristov et al., 1999). After recording the

Table 1 Chemical composition of *Leymus chinensis*, concentrate and mixed diet (g·kg^{-1} DM)

item	*Leymus chinensis*	concentrate[a]	mixed diet[b]
dry matter (DM)	883	860	871.5
organic matter (OM)	844	936	890
crude protein (CP)	80	167.4	123.7
soluble CP	48	126	87
NDF	707.4	298.6	503
ADF	426.4	303.6	365
calcium	4.8	7.6	6.2
phosphorus	3.6	4.6	4.1

Note: (a) Contained corn, maize, soybean and urea (10 g·kg^{-1}), $CaCO_3$ powder, dicalcium phosphate, salt, malt, vitamins premix (vitamin A, 100100 IU·100g^{-1}; vitamin D, 50000 IU·100g^{-1}; vitamin E, 1000 IU·100g^{-1}) and minerals ($FeSO_4·H_2O$, 23 mg·kg^{-1}; $CuSO_4·5H_2O$, 10 mg·kg^{-1}; $MnSO_4·H_2O$, 14 mg·kg^{-1}; $ZnSO_4·H_2O$, 17 mg·kg^{-1}; Na_2SeO_4, 0.05 mg·kg^{-1}; KI, 0.06 mg·kg^{-1} and $CoCl_2·6H_2O$, 0.16 mg·kg^{-1}). (b) 500 g·kg^{-1} concentrate and 500 g·kg^{-1}*Leymus chinensis* on DM basis.

pH of the filtrate, the subsamples of the filtrate were analyzed for VFA concentrations using GLC (model 3300; Varian Associates, Walnut Creek, CAI). To analyze ammonia, 30 mL subsamples of filtrate were added to 2.25 mL of 65% (wt/vol) trichloroacetic acid (TCA). The mixtures were kept on ice for 30 min and then centrifuged (28000 × g, 15 min, at 4°C). Supernatants were stored at −40°C until analysis. After the collection of samples, the additional subsamples of filtrate were frozen immediately for the analysis of protozoal populations by combining 2.5 mL of filtrate with 5 mL methyl green, formalin and saline (MFS) solution. Numbers of protozoa were counted using a Fuchs-Rosenthal counting chamber. We chose three subsamples from each filtrate, then selected five large squares randomly in each subsample. MFS preparation was counted.

2.3.2 Serum sampling and preparation

On days 18 and 19, serum samples were collected from the jugular vein 2 h after the morning feeding, and serum chemistries were analyzed by an auto blood-analysis machine (KX-21N, Japan). The measurements included Aspartate aminotransferase (AST), Glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), Creatinin (CRE) and Blood Urea-Nitrogen (BUN) assay.

2.3.3 Statistical analyses

Data were analyzed by PROC MIXED procedure of SAS (1996) for repeated measures. Significant differences between treatment means were determined by Duncan's multiple range test.

3 Results

3.1 Ruminal pH

The observations indicated that the acidity of ruminal fluid increased after feeding for all treatments (Table 2). From 0 to 6 h after feeding, the ruminal pH value was numerically higher in groups with YSE treatments than in the control group ($P = 0.08$). The average ruminal pH values (over all sampling times) in sheep varied from 6.50 to 6.67, within the optimum pH range to maintain normal cellulolytic organisms (6.7 ± 0.5, van Soest, 1994). Ruminal pH values in all treatment groups decreased soon after feeding, then reached the lowest point about 4–6 h after feeding. Ruminal pH values then started increasing and almost returned to the same level before feeding about 8 h after feeding. The pH showed some differences among the treatment groups, that is, as the level of YSE increased, pH decreased in time (Fig. 1). This effect helped improve the balance and stability of rumen microbes.

3.2 Ruminal VFA and the ratio of acetic acid to propionic acid

Results in Table 2 also show that the acetic acid concentration decreased, but the propionic acid values increased as the YSE concentration increased. The averaged acetic acid concentration of the 300 mg·kg^{-1} group was 17.5% lower than that of the control group ($P < 0.05$), and 12.5% lower than that of the 100 mg·kg^{-1} group ($P < 0.05$). However, the average propionic acid concentration in the 300 mg·kg^{-1} group was 29.8% higher than that of the control group ($P < 0.05$), and 21.6% higher than that of the 100 mg·kg^{-1} group ($P < 0.05$). In this

Table 2 Effect of YSE on rumen fermentation and protozoa populations

item	*Yucca schidigera* extract/mg·kg^{-1}				S.E.M.	effect[a] (*P*-value)		
	0	100	200	300		D	T	D × T
pH	6.67	6.63	6.50	6.55	0.024	0.13	0.08	0.64
ammonia N /mg·100mL^{-1}	18.66a	18.56a	16.01b	16.14b	1.452	0.03	0.17	0.53
VFA/mmol·L^{-1}	–	–	–	–	–	–	–	–
aceticacid	43.79a	41.28a	38.42b	36.12b	0.181	0.05	0.47	0.34
propionic acid	17.02b	18.16b	19.13b	22.09a	0.173	0.02	0.52	0.61
butyric acid	6.22	7.10	6.29	6.74	0.124	0.21	0.61	0.46
Iso-Acids[b]	0.41	0.36	0.47	0.39	0.091	0.38	0.77	0.68
total VFA	67.44	66.90	64.31	65.34	1.270	0.67	0.03	0.12
protozoa /10^4·mL^{-1}	11.86a	11.19a	10.72b	9.81b	0.978	0.02	0.11	0.34

Note: Different letters in the same row denote significant difference at $P < 0.05$. "a" means D, T and D × T represent diet, time and interaction between diet and time, respectively. "b" includes iso-butyrate, valerate and iso-valerate.

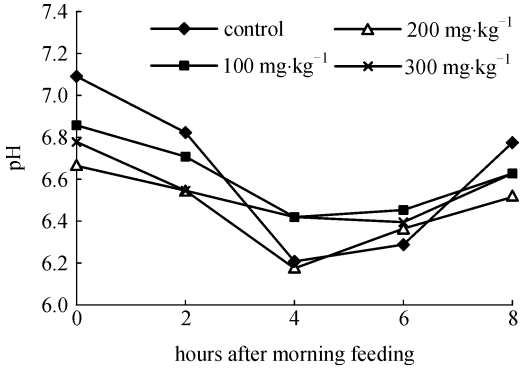

Fig. 1 Effects of YSE dietary treatments on pH variation at different time

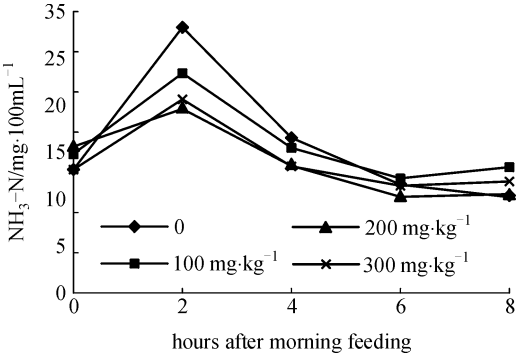

Fig. 2 Effects of YSE dietary treatments on NH_3-N variation at different times

study, the average TVFA concentration was found in the normal range (50–100 mmol·L^{-1}). The TVFA concentration in the four groups was not affected by YSE ($P = 0.67$), but by the sampling time ($P < 0.05$). The concentration peak of TVFA was observed 2–4 h after feeding. The averaged treatment data showed a regular trend, that is, TVFA concentration rose rapidly at a certain period after feeding until reaching its peak after about 4 hours, then stayed at a constant level until the occurrence of a quick going-down stage. The molar proportion of total iso-acids (iso-butyrate, valerate and iso-valerate) was not affected by either YSE ($P < 0.05$) or sampling time ($P = 0.77$).

3.3 Ruminal ammonia-N concentrations

In all treatments, ruminal ammonia-N concentrations increased fluctuantly towards the peak about 2 h after feeding (Fig. 2). Sheep receiving no YSE had a greater increase in ruminal ammonia concentration in the first 2 h after feeding (increased by 17.57 mg·100 mL^{-1}, $P < 0$ .05) than sheep receiving 200 mg·kg^{-1} YSE (increased by 6.77 mg·100 mL^{-1}) and 300 mg·kg^{-1} YSE (increased by 6.50 mg·100 mL^{-1}). After 4 h, the 200 and 300 mg·kg^{-1} groups still had lower ammonia concentrations than the control animals (decreased by 3.14 mg·100 mL^{-1}, $P < 0.05$ and decreased by 3.38 mg·100 mL^{-1}, $P < 0.05$, respectively). However, at 6 to 8 hours after feeding, no significant difference was found between the YSE groups. Table 2 shows that the 200 and 300 mg·kg^{-1} groups had a lower average ammonia concentration compared with the control group and the 100 mg·kg^{-1} group ($P < 0.05$).

3.4 Protozoal populations

The effects of YSE on the populations of protozoa in the sheep rumen (Table 2) showed a reduction, and the population fluctuated after feeding of the YSE treatment ($P < 0.05$). On average, protozoa in the ruminal fluid of sheep receiving 300 mg·kg^{-1} of YSE was 17.3% less ($P < 0.05$) than that of the control group, but no significant differences among the groups of 100, 200 mg·kg^{-1} of YSE and the control group was found.

3.5 Serum chemistries

As shown in Table 3, the serum traits did not change between the YSE treated groups and the control group. The serum traits were within the normal physiological range for sheep (Zhang, 2003).

Table 3 Effects of different levels of YSE on serum traits of sheep

item	YSE/mg·kg^{-1}						
	0	100	200	300	S.E.M.	*P*-value	normal physiological ranges for sheep
AST/IU·L^{-1}	84.67	75.67	85.33	76.67	4.12	0.23	50–150
GGT/IU·L^{-1}	38.00	35.00	43.67	37.33	6.21	0.43	31–60
ALP/IU·L^{-1}	121.33	116.67	119.73	103.67	11.23	0.31	80–140
LDH/IU·L^{-1}	64.67	67.00	73.00	61.67	4.43	0.27	40–93
CRE/μmol·L^{-1}	80.67	77.67	69.676	83.00	12.18	0.38	54–110
Urea-N/mmol·L^{-1}	8.33	8.03	7.53	6.80	0.82	0.15	2.1–9.6

4 Discussion

The decrease in ruminal pH value with YSE supplementation has been reported before (Hristov et al., 1999). Santoso et al. (2004) observed that sheep ruminal pH values were significantly altered by supplementing YSE at the 120 mg·kg^{-1} level of diet ($P < 0.05$). In our present study, ruminal pH of sheep receiving YSE was found to be slightly decreased, although the difference was not significant compared with the control animals ($P = 0.13$). A large amount of VFA was produced during the quick fermentation of the easily-degenerated nutrients in the rumen. We suggest that this was the reason for the rapid decrease in ruminal pH values within the 2 hours after feeding. YSE can enhance the surface active properties of rumen well, which helps VFA go through easily. In addition, YSE can also slow down the speed of urea's decomposition into ammonia, and delay the decline of pH (Pen et al., 2007).

The function of rumen fermentation was quite active after feeding in our study, which resulted in the increase in TVFA concentration and then decreased with the depletion of nutrients. Wang et al. (1998) reported that YSE has the bio-function of increasing acetic acid concentration and reducing the propionic acid concentration, but had no influence on TVFA concentration. We attribute the result to the improved multiplication and the balanced rumen microbes (such as protozoal populations) by adding YSE to sheep feed. This property limited the activities of negative factors so as not to produce propionic acids and to promote the activities of acetic acid-producing-microbes. Hence, the concentration of propionic acid increased.

Adding YSE can significantly reduce the protozoal populations in rumen, which promotes the activity of the propionic acid-producing-microbes during fermentation. Thus, the fermentation results in a high propionic acid level (Liu et al., 2005).

Ammonia binding by YSE has been shown in laboratory assays at high concentrations *in vitro* (Pen et al., 2007). Hristov et al. (1999) included YSE in diets for heifers and observed a significant decrease in ruminal ammonia concentration. In our present study, ruminal ammonia concentration of sheep reduced in all treatments compared with the control sheep after 4 and 6 hours ($P < 0.05$). YSE can bind ammonia by the glycolcomponent separated from the saponin fraction, which is toxic to rumen ciliate protozoa and inhibits some ruminal bacteria that could decrease deamination (van Soest et al., 1991). Muhammad et al. (2002) reported that YSE decreased ammonia production through restrained urease activity, which catalysed the reaction and resulted in the release of ammonia.

Protozoa can ingest and digest bacteria in the rumen, which can decrease the flow of microbial proteins from the rumen. If digestibility of dietary fiber is not adversely affected, this also wastes energy in the net synthesis of bacterial protein in the rumen. Therefore, reducing protozoal populations in cattle can improve nitrogen utilization in the rumen and increase microbial protein flow to the intestine. Animal performance can be increased by decreasing rumen protozoa. YSE has a strong antiprotozoal activity and may serve as an effective defaunating agent for ruminants. Its toxicity to protozoans seems to be widespread and nonspecific, which is the direct result of the YSE detergent effect on the cell membranes.

The activities of AST, GGT, ALP, LDH, and CRE can be used as parameters to assess functions of the kidney, pancreas, liver, spleen, intestine, brain, lung, skeletal muscle, cardiac muscle and other tissues clinically. No differences in serum traits were observed in the present study. The urea-N concentrations of sheep receiving 200 mg·kg^{-1} and 300 mg·kg^{-1} YSE were less than that of the control group. However, no significant difference with the group of 100 mg·kg^{-1} YSE was found ($P = 0.15$). Urea-N is the ultimate product of the metabolism of proteins, amino acids and other N-contained substances in mammals (Pen et al., 2007).Urea-N can reflect the utilizing efficiency of ration-N, and urea-N is also an important index reflecting liver and kidney functions because it is highly correlated to rumen-N. This experiment showed that urea-N was within a normal scope (2.1–9.6 mmol·L^{-1}). However, no significant changes in urea-N level were found by increasing the level of YSE. This result disagrees with the study of Killeen et al. (1998) that YSE can improve kidney function in urea-excretion and reduce ammonia concentration and urea concentration in serum.

5 Conclusions

This study suggests the possibility of manipulating ruminal fermentation through adding YSE in diets. The effect of YSE on ruminal ammonia concentration is most likely the result of a decreased concentration of protozoa, presumably, from ammonia binding by YSE. Within the 200–300 mg·kg^{-1} range of diet, the variety of serum traits in the groups was in a normal physiological range. This proves that these levels of YSE are safe for sheep.

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