Effects of the Administration of Yucca shidigera Saponins on Pigs Intestinal Microbial Population

Yu KATSUNUMA, Makoto OTSUKA, Yutaka NAKAMURA, Atsushi TOYODA, Ryozo TAKADA1 and Hajime MINATO

Faculty of Agriculture, Ibaraki University, Ami-machi, Ibaraki-ken 300-0393, Japan
1 National Institute of Animal Industry, Tsukuba Norin Kenkyu Danchi, Ibaraki-ken 305-0901, Japan

(Received June 22, 2000; Accepted September 13, 2000)

Abstract Three pigs were fed a diet supplemented with 50 ppm Yucca shidigera saponins, and the remaining three pigs were given a nonsupplemented diet. The feces of the pigs were collected at 103 days of age after birth. The concentration of ammonia-N in the feces of pigs given a diet supplemented with saponins was significantly lower than the pigs given the nonsupplemented diet. The total volatile fatty acids concentration in the feces was not significantly different between the pigs given a diet supplemented with or without saponins. However, the molar proportion of the acetic acid in the feces of pigs given a diet supplemented with saponins was lower, and inversely the concentration of the propionic, butyric and n-valeric acids was higher in comparison with the pigs given the non-supplemented diet. The total viable counts in the feces were not different between the pigs given a diet supplemented with or without saponins. Bifidobacteria, eubacteria, and staphylococci were more abundant in the feces of pigs given a diet supplemented with saponins, as compared with the pigs given the non-supplemented diet. Veillonella was less abundant in the feces of pigs given a diet supplemented with saponins than the pigs given the non-supplemented diet.


Key words: Yucca shidigera saponins, Intestinal bacteria, Bifidobacteria, Veillonella

The Yucca shidigera plant belongs to the family, Agavaceae, and grows in the southwestern deserts of the United States and in the Baja California region of Mexico. The native populations of the semi-arid deserts of the Americas have used the extract of Y. shidigera plants as therapeutic agents for many kinds of diseases for many years. More recently, extracts of the Y. shidigera plant have been used extensively as flavouring and foaming agents in the food and soft drink industry, in cosmetics for their surfactant property and in the feed industry7). Especially, extracts of Y. shidigera plant are used as feed additives for animals and poultry, to improve animal and poultry production performance, including weight gain, feed efficiency, and health, and to reduce the emission of ammonia from animal excreta6,10,13,14).

Extracts of Y. shidigera are rich in saponins, which consist mainly of steroidal saponins such as sarsasaponin and smilagenin. Saponins have been proved to have a number of pharmaceutical effects, including enhanced vaccine effectiveness and antitumor and antimicrobial activity19). In addition, saponins have exhibited an ability to reduce plasma cholesterol in a number of mammalian species, including humans and animals6).

In a previous paper we reported the effects of Y. shidigera extract and saponins on the growth of bacteria isolated from the intestinal tract of animals12). Namely, the growth of anaerobes, often isolated from clinical specimens of humans and animals, was inhibited by Y. shidigera extract and saponins. On the other hand, the Y. shidigera extract and saponins did...
not inhibit the growth of *Lactobacillus* spp. and *Bifidobacterium* spp., which play an important role in animal health. Now, it is confirmed that saponins in *Y. shidigera* extract are the major components responsible for its antibacterial and antifungal activity\(^\text{12,17,21,22}\). However, no information exists concerning the effect of *Y. shidigera* saponins on the microbial constitution in the intestinal tract of animals.

Therefore, in the present study the effects of *Y. shidigera* saponins on the volatile fatty acids (VFA) concentration and proportion, the ammonia-N concentration, and the constitution of microbial populations in the intestine of pigs were investigated.

**Materials and Methods**

*Yucca shidigera saponins*

The *Yucca shidigera* saponins used in this investigation were Yucca Sarsaponin 80-M (Lot No. 980305), which was supplied by Mitsuba Trading Co., Ltd., Tokyo, Japan and contained 90.9% saponins.

**The animals and their management**

A litter of pigs (Duroc × Yorkshire × Landrace) was used in this investigation. The pigs were reared on farms at the National Institute of Animal Industry (Kukizaki-machi, Ibaraki). The pigs were breast-fed after birth, and weaned at 4 weeks of age. Six pigs weighing 6.5 to 7.6 kg, were selected and housed in separate pens. The pigs were offered a commercial formula feed (‘Mama 7 Pawafuruakuto’, Kyodo Shiryo, Co., Ltd., Kanagawa) for the first week postweaning, and subsequently given another commercial formula feed (‘Mama 8 Pawafurukoki’, Kyodo Shiryo, Co., Ltd., Kanagawa) for 5 weeks. Six pigs were assigned to two experimental groups at 10 weeks of age. Each experimental group consisted of one male and two females. Three pigs were offered ad libitum a diet that contained *Y. shidigera* saponins at a concentration of 50 ppm. The remaining three pigs were given ad libitum a diet without saponins. The control diet used in this feeding experiment was a self-formulated one for pigs in the growing phase, which contained mainly corn and soybean meal (Table 1). And, the experimental diet used in this experiment contained 0.0055% Sarsaponin 80-M in addition to the control diet.

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn</td>
<td>37.95</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>41</td>
</tr>
<tr>
<td>Dried whey</td>
<td>15</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.7</td>
</tr>
<tr>
<td>Calcium phosphate</td>
<td>1.4</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.3</td>
</tr>
<tr>
<td>Corn oil</td>
<td>3</td>
</tr>
<tr>
<td>Trace mineral premix(^1)</td>
<td>0.15</td>
</tr>
<tr>
<td>Vitamin A, D, E premix(^2)</td>
<td>0.15</td>
</tr>
<tr>
<td>Vitamin B premix</td>
<td>0.15</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>0.1</td>
</tr>
<tr>
<td>D, L-Methionine</td>
<td>0.1</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>10 mg/kg</td>
</tr>
<tr>
<td>Vitamin B(_12)</td>
<td>15 (\mu)g/kg</td>
</tr>
</tbody>
</table>

1) Trace mineral premix contained the following in grams per kilogram: manganese sulfate 137.5, iron sulfate 136, copper sulfate 25.1, zinc carbonate 115.2, calcium iodate 1.54.

2) Vitamin premix contained the following per kilogram: vitamin A 15000 I. U., vitamin D\(_3\) 3000 I. U., dl-\(\alpha\)-tocopherol 10 mg, thiamin 1 mg, riboflavin 7 mg, pyridoxine hydrochloride 0.5 mg, nicotinamide 6 mg, D-calcium pantothenic acid 10.9 mg, choline chloride 57.6 mg.

**Sampling of fresh feces**

After 5 weeks from the start of feeding the experimental diets, fecal samples were obtained directly from the rectum of the pigs before morning feeding.

**Culture media and microbiological methods**

One gram of the fecal sample was blended under 100% CO\(_2\) in 9 ml of anaerobic dilution solution containing 0.05% agar\(^3\). Further serial dilutions were made in an anaerobic dilution solution for bacterial enumeration.

The total viable counts of anaerobes were determined by the anaerobic roll tube method described by Hungate\(^5\). A modified M-10 medium containing 5% autoclaved clarified swine cecum fluid, was used to count the total viable anaerobes.

The differential viable counts of both anaerobes and aerobes were made according to the method and
Table 2. Concentration of ammonia-N in the feces of pigs given diets supplemented with or without *Y. shidigera* saponins

<table>
<thead>
<tr>
<th></th>
<th>Diet supplemented with saponins (n = 3)</th>
<th>Diet without saponins (n = 3)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia-N concentration (mg/100 g · feces)</td>
<td>81.3±1.2</td>
<td>110.3±0.8</td>
<td>***</td>
</tr>
</tbody>
</table>

* Mean and standard error of the mean.
*** Significant difference at the level of 1%.

The agar plates inoculated for counting the aerobes were aerobically incubated at 37°C for 2 days. Five kinds of media-DHL agar, TATAC agar, PEES agar, TS agar, and P agar—were used for counting the aerobic microorganisms. The agar plates inoculated for counting the anaerobes were incubated anaerobically in an anaerobic jar at 37°C for 2 days. Twelve kinds of selective and nonselective media-ES agar for eubacteria; LBS agar for lactobacilli; BS agar for bifidobacteria; VS agar for veillonella and mega-sphaera; NN agar for *Clostridium perfringens*; NBGT agar for bacteroides; FS agar for fusobacteria; PO agar, RCN agar and PNC agar for clostridia; BL agar and EG agar for total anaerobe counts—were used for counting anaerobic bacteria.

**Analytical methods**

The ammonia-N concentration in the feces was determined by the colorimetric method according to Kaplan’s phenate-hypochlorite method. Two grams of feces were weighed into a test tube, and 8 ml of distilled water was added. The sample was homogenized on a Vortex mixer and centrifuged at 8,000 × g (10,000 rpm) for 10 min. The supernatant was used for the determination of ammonia-N concentration.

The VFA concentration in the feces was analyzed by gas chromatography according to Minato et al. The pretreatment of the feces for VFA analysis was performed according to the procedures described by Minato et al.

**Statistical analysis**

Data was evaluated by using a Student’s t test at three significant levels.

---

**Results and Discussion**

**Ammonia-N concentrations**

Ammonia-N concentrations in the feces were compared between the pigs given a diet supplemented with a 50 ppm concentration of *Y. shidigera* saponins and the pigs given the non-supplemented diet, and the obtained results are shown in Table 2. The ammonia-N concentrations in the feces of pigs given a diet supplemented with saponins were significantly lower than those given the non-supplemented diet. However, the reduction of ammonia-N concentrations in the feces of pigs given a diet supplemented with saponins was only 26%, as compared with pigs given the non-supplemented diet.

High ammonia concentrations in animals and poultry houses have long been recognized as having a negative effect on the health and performance of poultry, pigs and calves. Atmospheric ammonia has been regarded as one of the major causes of the pathogenesis of respiratory tract diseases in animals and poultry. In addition, ammonia has been shown to have negative effects on a variety of biological systems including metabolic hormones and reproduction. The obtained results in this investigation suggest that *Y. shidigera* saponins, as a feed additive, provides good effects on the health and performance of pigs through the reduction of ammonia concentrations in the feces.

Recent experiments have demonstrated that extracts of *Y. shidigera* had ammonia-binding properties. The fractionation of *Y. shidigera* extracts by treatment with n-butanol, which removes the saponins, has shown that the ammonia-binding property is not associated with the saponins, but are
Table 3. Concentration and composition of VFA in the feces of pigs given diets supplemented with or without
*Y. shidigera* saponins

<table>
<thead>
<tr>
<th></th>
<th>Diet supplemented with saponins (n = 3)</th>
<th>Diet without saponins (n = 3)</th>
<th>r-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total VFA concentration (mM/100 g · feces)*</td>
<td>5.6±1.0</td>
<td>6.2±0.4</td>
<td>ns</td>
</tr>
<tr>
<td>VFA composition (mol%)*</td>
<td>Acetic acid: 48.2±4.3</td>
<td>61.9±1.4</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Propionic acid: 27.6±3.6</td>
<td>21.8±2.0</td>
<td>*</td>
</tr>
<tr>
<td></td>
<td>Isobutyric acid: 4.4±2.8</td>
<td>4.6±0.2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Butyric acid: 10.2±0.4</td>
<td>7.4±1.0</td>
<td>***</td>
</tr>
<tr>
<td></td>
<td>Isovaleric acid: 2.3±0.3</td>
<td>2.4±0.2</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Valeric acid: 7.4±2.7</td>
<td>2.0±0.3</td>
<td>**</td>
</tr>
</tbody>
</table>

VFA: volatile fatty acids.

* Mean and standard error of the mean.

** Significant difference at the level of 1%.

VFA concentrations and proportions

The VFA concentrations and molar proportions in the feces of both pigs given a diet supplemented with saponins and the pigs given the nonsupplemented diet are shown in Table 3. The total VFA concentration in the feces of pigs given a diet supplemented with saponins was not significantly different from that of pigs given the nonsupplemented diet. And, inversely, that of propionic, n-butyric and n-varelic acids in the feces of the pigs given a diet supplemented with saponins was higher when compared with the pigs given the nonsupplemented diet. Volatile fatty acids (VFA) is mainly produced from carbohydrates and proteins by microbial degradation. Therefore, this significant difference in VFA molar proportions in the feces between the pigs fed a diet supplemented with saponins and the pigs given the nonsupplemented diet may reflect the difference in the constitution of the microbial populations in the intestine.

Microbial populations

The total viable counts of anaerobes and the constitutions of microbes in the feces of both pigs fed a diet supplemented with saponins and the pigs given the nonsupplemented diet are shown in Table 4. The total viable counts of anaerobes in the feces of pigs fed a diet supplemented with saponins were not different from those of the pigs given the nonsupplemented diet. On the other hand, there were several distinct differences in the constitutions of the microbial populations in the feces between the pigs fed the diets supplemented with or without saponins.

Viable counts of bifidobacteria, eubacteria and staphylococci were significantly higher in the feces of pigs fed a diet supplemented with saponins than the pigs given the nonsupplemented diet. Inversely, the viable count of mesosphaera in the feces of pigs given a diet supplemented with saponins was significantly lower as compared with the pigs given the nonsupplemented diet.

Bifidobacteria are the predominant bacteria in the animal intestinal tract and play an important role in animal health\(^2\). Therefore, some kinds of lactic acid bacteria and bifidobacteria have been used as probiotics in animal production\(^1\). The results obtained in this investigation showed that bifidobacteria were more abundant in pigs given a diet supplemented with saponins as compared to the pigs given the nonsupplemented diet. While, veillonella are often isolated from clinical specimens of human and animals\(^5\). As shown in the previous study, the growth of \textit{E. aerofaciens} was inhibited by \textit{Y. shidigera} saponins\(^12\). However, it was not examined whether the growth of other \textit{Eubacterium} species is inhibited by \textit{Y. shidigera} saponins or not. The results obtained in this investigation showed that eubacteria were more abundant in the feces of pigs given a diet supplemented with saponins when compared to the pigs given the nonsupplemented diet. While, the molar proportion of \textit{n}-butyric acid in the feces of pigs, which were fed a diet supplemented with saponins, was much higher as compared with the pigs given the nonsupplemented diet. \textit{E. aerofaciens} do not produce \textit{n}-butyric acid as end products from carbohydrates, but most \textit{Eubacterium} species are able to produce \textit{n}-butyric acid as end products\(^8\). On the basis of these facts, it is assumed that eubacteria except \textit{E. aerofaciens} became abundant in the feces of pigs fed the diet supplemented.
Yucca Saponins and Intestinal Microbial Population

with saponins and consequently the concentration n-butyric acid in the feces increased.

The results obtained in this investigation show that Y. shidigera saponins affect the constitution of the intestinal microbial population of pigs.

Acknowledgments

This research was financially supported by research grants from the Ministry of Agriculture, Forestry and Fisheries (1999) and from the Ito Foundation, Tokyo, Japan.

References