Toxicological Investigations of Two Poisonous Plants, Tansy Ragwort (*Senecio jacobaea*) and Summer Dandelion (*Hypochaeris radicata*) with Potential Therapeutic Agents, Quillaja and Milk Thistle

By
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Chapter 1:

Investigation of a Chicken Model for Equine Stringhalt Caused by Summer Dandelion (*Hypochoeris radicata*)
Investigation of a Chicken Model for Equine Stringhalt Caused by Summer Dandelion (*Hypochaeris radicata*)

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**Abstract**

Stringhalt is a disorder in horses characterized by high stepping with hyperflexion of the hind limbs. This disorder has been reported from the US, Australia, Chile, and Brazil. In most instances, the disease manifests itself in horses exposed to summer dandelion (*Hypochaeris radicata*). Stringhalt has been induced by feeding summer dandelion to a horse, thus directly implicating the plant. The causative agent or agents in the plant have not been identified. Characterization of a small laboratory animal model which would exhibit neuromuscular clinical signs and/or lesions when fed dandelion would greatly facilitate further studies to identify the active principle in *Hypochaeris radicata*. In this study immature Rhode Island Red hybrid chicks were used as the animal model. Because chickens are bipedal, it was hypothesized that any signs of muscular degeneration affecting locomotion would be obvious. The chicks were divided into three treatment groups; the control, a 10 weight percent summer dandelion diet, and a 30 weight percent summer dandelion diet. Feed intake was measured daily and weights were recorded weekly. The chicks were fed this diet for 35 days, then humanly euthanized and necropsied. Heart, liver, spleen, leg muscles, the femoral and sciatic nerve, spinal cord and brain were taken for histopathology. There were no statistical differences in body weight, feed consumption, liver, or heart muscle weights between the different treatment groups.
Introduction

Stringhalt is a disorder in horses characterized by high stepping with hyperflexion of the hind limbs. The disorder has been reported in the US, Australia, New Zealand, Chile, and Brazil (Burrows and Tyrl, 2013). In most instances, the disease is a neurological disorder manifest in horses grazing on poor quality pastures where summer dandelion (*Hypochaeris radicata*) is the predominant available forage (Gardner 2005). Stringhalt was induced in a horse by feeding 490 kg summer dandelion (Arauju et al. 2008), thus directly implicating the plant. The causative agent or agents in the plant have not been identified. While the horse is the target animal involved, the amount of material (plant, extracts, or pure compounds) required to characterize the active agent precludes using the horse as the animal model. Identification of a small laboratory animal model which exhibits neuromuscular clinical signs or lesions when fed dandelion would greatly facilitate identification of the active principle in *Hypochaeris radicata*.

Pathological lesions of stringhalt in horses reported in the literature include axonal degeneration, particularly of the larger myelinated fibers, neurogenic muscle atrophy, and segmental demyelination (Cahill et al. 1986). Chicks were used as the animal model in this study. Because chickens are bipedal, any signs of muscular degeneration should produce very obvious signs of difficulty in locomotion.

The objective of this study was to determine if chicks would exhibit neurological signs or muscle impairment when fed summer dandelion.
Materials and Methods

Plant material- Hypochaeris radicata plants were collected near Corvallis, Oregon in 2010 and 2011. Above-ground parts of the flowering plants (leaves, stems, flowers) were collected and sun dried. The dried plants were finely ground for incorporation into the experimental diets.

Dietary Treatment- Three different treatment groups, consisting of varying amounts of the summer dandelion, were developed.

1) 0 weight percent summer dandelion
2) 10 weight percent summer dandelion
3) 30 weight percent summer dandelion

The ground summer dandelion was mixed in with Intermountain Farmers Association’s medicated 20% chick starter mash, code #1154, at the designed concentration.

Animals- 18 Rhode Island Red hybrid chicks were purchased from the Intermountain Farmers Association Country Store in Logan, UT. Three males and three females were randomly assigned to each treatment group. The chicks had food and water readily available throughout the entire experiment. Body weight was determined before feeding and once a week during the experimental feeding period. The feed intake data was measured daily. At the end of the 35 day feeding period, all animals were humanely euthanized and necropsied. Organ weights, including the liver and heart, were taken during necropsy. Liver, heart, lung, kidney, spleen, leg muscles, femoral and sciatic nerve, upper and lower spinal cord, and the brain were all obtained for histological examination. All protocols for animals used in this research were performed under veterinary supervision and approved by the Institutional Animal Care and Use Committee (IACUC), Utah State University, Logan, UT.
Results and Discussion

Behavioral observations throughout the feeding period showed no obvious differences between the treatment groups. All chicks appeared healthy throughout. Feed intake was measured and recorded daily (figure 1). The amount of feed consumed by each treatment group was not statistically different between control and treatment groups ($p > 0.05$). Chicks were weighed weekly (figures 2 and 3), and statistical analysis completed on the weights recorded the final two weeks of the study, the “final weights”. There was no difference in average weights between the control and 10% summer dandelion ($p > 0.05$), whereas there was a significant difference between the control and the 30% summer dandelion treatment groups ($p = 0.002$).

At necropsy, sections of brain, spinal cord, sciatic nerve, quadriceps femoris and biceps femoris, heart, lung, liver, spleen, pancreas, proventriculus, ventriculus, duodenum, ileum cecum, colon, and testes were examined. The liver (figure 4) and heart weights were recorded during necropsy and there were no differences between controls and treatment groups ($p > 0.05$). One of the chicks that consumed the 10% summer dandelion diet had liver lesions and an overall darkening of the liver. Two of the chicks in the 30% summer dandelion treatment group showed similar lesions and darkening. One of the chicks in the control group also had a dark, soft liver. Histological examination showed no significant lesions in any of the collected tissues from any of the chicks.

This study suggests that chicks do not manifest neurological effects or neuromuscular lesions when summer dandelion is fed at the doses or duration reported in this experiment. Further research is required to more fully characterize the potential use of the chick model for summer dandelion induced stringhalt research.
Figure 1. Comparison of average feed intake (g) of each chick per day by treatment group over the experimental feeding period.

Figure 2. Comparison of average final body weights (g) of each treatment group throughout the experimental feeding period. The “final” weights were determined using the average of the last two weighings.
Figure 3. Comparison of weekly average body weights (g) of chicks in each treatment group over the experimental feeding period.

Figure 4. Comparison of liver weights, displayed as a percentage of the final body weight, of chicks in each treatment group over the experimental feeding period.
**Conclusion**

The results of this trial suggest that over a 35 day feeding period, 10 and 30 weight percent summer dandelion diets do not induce stringhalt in chicks used as an animal model. An increased feeding period is required to fully understand the extent to which chickens may be used as an animal model for stringhalt.
Sources


Chapter 2:

The Effects of the Administration of Milk Thistle on Pyrrolizidine (Senecio) Alkaloid Toxicity in Chicks
The Effects of the Administration of Milk Thistle on Pyrrolizidine (*Senecio*) Alkaloid Toxicity in Chicks

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Abstract

*Silybum marianum*, commonly known as milk thistle (MT), is reputed to be beneficial to liver health. The active ingredient in MT is silymarin, the biologically active ingredient from the MT seed. Dietary supplements of MT usually 70-80% silymarin, can be purchased at common herbal supplement and health food stores, with recommendations that taking one to three capsules a day (200 mg of material per capsule, 80% silymarin) may speed the regeneration of injured liver cells, and support, protect, and improve function of liver cells. The objective of this study was to determine if MT would reduce the toxic effects of pyrrolizidine alkaloids (PA) from tansy ragwort (TR) (*Senecio jacobaea*), including damage to the liver, kidneys, heart, smooth muscles, and lungs. Chicks were randomly assigned to one of four treatment groups, with or without MT paired with two different concentrations of TR (0 and 5 weight percent). TR was mixed into the feed at the designed concentration and MT was administered twice daily via gavage. Feed intake, body weights and clinical signs were recorded throughout the study. Many of the chicks that had consumed the TR began to show signs of lethargy, loss of appetite, and weight differences before the end of the feeding trial. Two of the six chicks that had consumed the TR diet without MT, died before the end of the feeding period, on days 42 and 45. All chicks were humanely euthanized 47 days after the start of experimental feeding and necropsied. At necropsy multiple chicks showed extensive liver damage, ascites, edema, enlarged spleen and low hematocrit. There was no
difference in clinical effects or gross pathology between chicks receiving MT and TR when compared to those receiving only TR in their diet. Histological examination of the livers showed no differences between groups receiving MT and TR and those receiving only TR in their diet. Based on the results of this study, MT does not provide any level of protection TR induced liver disease.

Keywords: Milk thistle, silymarin, tansy ragwort, pyrrolizidine alkaloid, liver

Introduction

Studies suggest that *Silybum marianum*, commonly known as milk thistle (MT), is beneficial to liver health. The active ingredient in the MT seed is silymarin (Foster and Tyler 1999). Milk thistle capsules, (70-80% silymarin), can be purchased at common health food and herbal stores. Promotional literature suggests that taking one to three capsules a day may speed the regeneration of injured liver cells, and support, protect, and improve function of liver cells (NIEHS 1998).

The effect of orally administered MT on the toxic potential of pyrrolizidine alkaloids (PA) was assessed in this study. The PA source was tansy ragwort (*Senecio jacobaea*). Tansy ragwort (TR) is distributed throughout Western Europe, South Africa, Australia, New Zealand, South America, the United States, and Canada. The PA’s are toxic to vertebrates and insects. Cattle and horses are particularly susceptible, and can die from hepatic failure due to PA toxicity (pyrrolizidine alkaloidosis). Pyrrolizidine alkaloidosis causes liver damage, including an enlarged, hemorrhagic, and/or icteric liver. The alkaloids themselves are not toxic, but the metabolic transformation in the liver to bioactive pyrroles is responsible for the toxic effects (Cheeke 1989). Because of the relative unpalatability, cattle usually avoid the plant unless other forage sources are scarce or when the plant included in harvested hay (USDA 2011).
The extent of liver damage caused by TR is hard to measure, although liver enzymes may be elevated for a period of time. An animal may consume a single large dose or multiple small doses of the plant without any outward appearance of toxicosis for many months. However, because of the irreversible and cumulative effects of the toxins on the liver, the animal may suddenly become sick and die. Because of the delayed effects of TR, the cause of death is often misdiagnosed (USDA 2011). In Oregon alone, TR can cause up to $5 million dollars in damage to farmers, including cattle and horse losses, increased herbicide use, and decreased pasture productivity (Coombs 1996).

The purpose of this study was to determine if orally administered MT would reduce or eliminate the hepatotoxic effects of the PA’s from TR in a chick model. According to label and literature claims, MT maintains liver health. Based on this information it was hypothesized that MT would reduce liver damage when co-administered to animals with PA’s.

**Materials and Methods**

**Plant material**- TR plants were collected in 2011 from Corvallis, Oregon. The flowers were solar dried and finely ground in a Wiley Mill through a 2 mm screen for use. The MT was purchased in capsule form from a General Nutrition Centers (GNC) store in Logan, Utah. Each capsule contained 200 mg of material, 80% (160 mg) of which is the silymarin extract from the seed. The other 20% (40 mg) is made up of cellulose, vegetable cellulose capsule, magnesium stearate, and corn starch. Only the material inside the capsule was used.

**Animals**- 24 Rhode Island Red hybrid chicks were purchased from the Intermountain Farmers Association Country Store in Logan, Utah. Three males and three females were randomly assigned to each treatment group, with the exception of the control group, which had five males and one female.
The chicks had food and water readily available throughout the entire experiment. Body weight was determined before feeding began and twice a week during the experimental feeding period. The feed intake data was measured daily. Noticeable changes in the chicks, such as lethargy, decreased appetite, and changes in behavior were documented. At the end of the 47 day experimental period, the animals were humanely euthanized and necropsied. All animals were necropsied with the liver, heart, and spleen weights measured against the control organs. Blood samples were also collected. Liver sections were obtained for histological examination for signs of PA toxicosis, such as centrilobular necrosis, megalocytosis, karyomegaly and/or fibrosis, in order to assess the effects of MT on the toxicity of hepatoxic PA’s from TR. All protocols for animals used in this research were performed under veterinary supervision and reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Utah State University, Logan, UT.

**Feeding**- The treatment groups for the study were as follows:

1) 5% TR feed +200 mg MT per day
2) 0% TR feed + 200 mg MT per day
3) 5% TR feed + 0 mg MT per day
4) 0% TR feed + 0 mg MT per day

The TR was mixed in with Intermountain Farmers Association’s medicated 20% chick starter mash, code #1154. The MT was administered by taking apart the MT capsule and emptying the contents into a test tube already that already contained 2 mL of water. After all of the capsule’s content was emptied, another 2 mL of water was put into the test tube. The test tube was then thoroughly shaken until the contents were evenly distributed and about half of the solution (~2 mL of water and 100 mg of capsule contents) was taken into a syringe with a blunt crop needle attached. Ensuring that there was no air in the needle, the needle was then lubricated with water and administered to the chick by oral gavage.
Half of solution was administered in the morning and the remaining half was administered in the afternoo...n. Treatment groups one and two were dosed every day.

**Results and Discussion**

Early in the experimental feeding, it was observed that the chicks consuming the TR mixed ration were not eating as much as the chicks on the control diet. The chicks that received the TR mixed diet consumed about half the amount of the ration as chicks fed ration without the TR (figure 1). Figure 2 shows the average amount of PA’s consumed by each chick per day, for each treatment group. GC-MS and LC-MS analysis indicated 2.19 mg of PA per gram of TR. The overall appearance of the chicks that did not consume the TR diet was much better compared to the chicks that did consume the TR diet, which was reflected in the weight gains (figure 3). The chicks were weighed twice every week. Based on the “final weights”, the average of the last two weighings, there was a significant difference in weights between chicks fed a diet containing TR vs. controls (p<0.05) but there was no significant difference (0% TR p-value= 0.20 and 5% TR p-value= 0.64) between the chicks consuming the same amount of TR with and without the MT. Beginning on day 40 of experimental feeding, some of the chicks began to appear sickly. One of the chicks that had consumed the TR without MT diet began to show signs of lethargy and decreased movement around day 40 and later died on day 42. Another chick in the same treatment group showed similar signs and later died on day 45 of experimental feeding. During the last week of feeding, the chicks that had consumed the TR began to look “puffed-up”, and lethargic. Some of the chicks in this same treatment group also appeared to have expanded, soft crops. All of the chicks were humanely euthanized on day 47 of experimental feeding.
At necropsy, liver, heart, and spleen weights were recorded. The chicks that had not consumed the TR showed no irregular gross observations during necropsy. The chicks that had consumed the TR, with or without the MT, showed small, pale, grandular livers, enlarged gall bladders, ascites, jaundice and occasional peritonitis. The weights of the spleen and liver, as a percent of final body weight, are in figures 4 and 5 respectively. The heart and liver organs did show significant results between the treatment groups not consuming the TR, with and without the MT (p-value= 0.04 and 0.01, respectively). The treatment group consuming both the TR and MT showed no significant difference between organ weights with and without the MT. Histological examination of liver tissue revealed no differences in the severity of lesions between groups that received TR with and without MT. The tissues were subjectively graded and scored on levels of hepatocellular swelling, hepatocellular necrosis, biliary proliferation and inflammation, where 0= no change, 1= minimal change, 2= mild to moderate change, and 3= severe change. Table 1 shows the rating of these effects in the treatment groups that consumed the TR. None of the ratings between the TR with and without the MT are significantly different.

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<th>5% Tansy Ragwort</th>
<th>5% Tansy Ragwort + MT</th>
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<td>Hepatocellular Swelling</td>
<td>2.0 +/- 0.6</td>
<td>2.1 +/- 0.8</td>
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<tr>
<td>Hepatocellular Necrosis</td>
<td>1.7 +/- 0.9</td>
<td>1.2 +/- 1.3</td>
</tr>
<tr>
<td>Biliary Proliferation</td>
<td>1.4 +/- 0.9</td>
<td>1.2 +/- 1.5</td>
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<tr>
<td>Inflammation</td>
<td>0.8 +/- 0.4</td>
<td>0.3 +/- 0.5</td>
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Table 1. Scored levels of severity or signs of liver toxicity, where 0= no change, 1= minimal change, 2= mild to moderate change, and 3= severe change.
Figure 1. The average feed consumed by each chick per day for each treatment group over the experimental feeding period.

Figure 2. Comparison of the average pyrrolizidine alkaloid (mg) consumption from tansy ragwort per kg body weight per day.
Figure 3. Comparison of average final body weights (g), determined by the average of the last two weighings, of each chick in each treatment group over the experimental feeding period.

Figure 4. Comparison of average spleen weights, as a percentage of the final body weight, of each treatment group.
Conclusion

This study suggests that oral administration of 160 mg of silymarin from MT does not prevent or reduce pyrrolizidine alkaloid toxicity from TR in chicks. There were no differences in incidence or severity of gross or microscopic lesions in the livers of chicks on TR diets with or with MT. Similarly, there were no differences in behavioral observations i.e., feed consumption or weight gains. Gross observations at necropsy, were also similar in chicks fed TR diets with or without the MT.
Sources


Binghamton, New York.


Chapter 3:

Toxicological Interactions Between Quillaja Saponins and Pyrrolizidine (Senecio) Alkaloids in Rats
Toxicological Interactions Between Quillaja Saponins and Pyrrolizidine (*Senecio*) Alkaloids in Rats

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Abstract

Saponins are glycosides found in plants, with particularly high concentrations in *Quillaja saponaria*, a tree native to Chile. Saponins have proven useful as dietary feed additives in agriculture. Saponins affect the permeability of intestinal cells by forming addition complexes with sterols in mucosal cell membranes, increasing the permeability of intestinal mucosal cells. The objective of this study was to determine if the saponins from *Quillaja saponaria* would alter the toxic effects of pyrrolizidine alkaloids (PA) from tansy ragwort (TR) (*Senecio jacobaea*), which includes damage to the liver, heart, and spleen. Rats were randomly assigned to one of six treatment groups, each receiving a different diet made up of varying concentrations of TR (0, 2.5, and 5 weight percent) and Quillaja powder (0 and 500 ppm). Feed intake and body weights were measured throughout the study. Many of the rats consuming the TR began to show signs of jaundice, decreased appetite, weight loss, and lethargy during the treatment period. Two of the 36 rats, both having consumed the 5 weight percent TR diet without Quillaja, died before the end of the feeding period, on days 44 and 47. The rats were necropsied 48 days after the start of experimental feeding. Necropsy showed extensive liver damage, ascites, edema, enlarged spleen, and low hematocrit in some of the rats that had consumed the TR diet, but showed no obvious difference between groups consuming and not consuming the Quillaja saponin. Further histological examination of the livers showed no difference in the TR pyrrolizidine alkaloid toxicity with or without
the Quillaja present in the diet. Results show that a dietary Quillaja concentration of 500 ppm does not influence the toxicity of PA to rats.

Keywords: quillaja saponaria, saponins, tansy ragwort, pyrrolizidine alkaloid

Introduction

Saponins are glycosides found in a variety of plants, with particularly high concentrations in *Yucca schidigera* and *Quillaja saponaria*, both desert plants native to Mexico and Chile respectively. Saponins have strong detergent and foaming properties, leading to their industrial applications in soft drinks, shampoos, and soaps (Cheeke 2001). Along with their commercial uses, saponins have also proven useful as dietary feed additives for livestock. Adding saponins to the diet has shown lowered serum cholesterol levels, improved rumen fermentation due to the inhibition of protozoa, and anticancer activity (Özlem 2007).

Saponins affect the permeability of intestinal cells by forming addition complexes with sterols (e.g. cholesterol) in mucosal cell membranes, increasing the permeability of intestinal mucosal cells, inhibiting active nutrient transport, and facilitating the uptake of substances to which the gut would normally be impermeable (Johnson et al. 1986). These findings were confirmed in a more recent study (Gee et al. 1997), in which saponins increased the transmucosal uptake of milk allergen β-lactoglobulin in rats. Saponin-exposed rats developed antigen-specific antibody responses to administration of ovalbumin (Atkinson et al. 1996), indicating that saponins may increase the sensitivity of animals to dietary antigens. A purified *Quillaja* (Q) saponin was effective as an agent to enhance absorption of orally administered drugs (Chao et al. 1998). Saponins from various food sources, such as oats (Onning et al., 1996) and quinoa (Gee et al. 1993), increase intestinal cell permeability.
The effect of dietary Q saponins on the toxic potential of pyrrolizidine alkaloids (PA) was assessed in this study. The PA source was tansy ragwort (*Senecio jacobaea*). Tansy ragwort (TR) is distributed throughout Western Europe, South Africa, Australia, New Zealand, South America, United States, and Canada. The PA’s are toxic to many vertebrates. Cattle are particularly susceptible, and can die from hepatic failure due to PA toxicity (pyrrolizidine alkaloidosis). Pyrrolizidine alkaloidosis caused by TR is known to cause liver damage, including an enlarged, hemorrhagic, and/or icteric liver. The alkaloids themselves are not toxic, but are metabolically transformed in the liver to bioactive toxic pyrroles (Cheeke 1989). Because of the unpalatable bitter taste, cattle usually avoid the plant (USDA 2011). TR may be consumed during times of drought when other food sources are scarce or when the plant is harvested in hay or mixed in feed. The Q saponins are widely used as animal feed additives; therefore, it is of importance to determine if dietary saponins affect the absorption of toxins in feeds, forages, and poisonous plants.

The Q saponins increase the permeability of intestinal cells. This could potentially change the absorption of toxins in feeds, forages, and poisonous plants. This change could have one of three results:

1. The Q saponins have no effect on the PA toxicity.
2. The Q saponins increase PA toxicity.
3. The Q saponins reduce PA toxicity.

In case two, an increase in large PA molecule absorption by the Q saponins will intensify the pyrrolizidine alkaloidosis, resulting in greater damage to the liver, kidneys, brain, heart, smooth muscles, lungs and DNA (Özlem 2007). In case three however, an interaction between the Quillaja saponins and bile acids may reduce the absorption of PA’s, lessening the effects of the pyrrolizidine alkaloidosis. Saponins bind with bile acids, reducing the absorption of lipid soluble substances (Oakenfull and Sidhu 1989).
The objective of this study was to determine if dietary Q saponins affect the toxicity of the poisonous plant, *Senecio jacobaea*, which contains hepatoxic pyrrolizidine alkaloids, in rats.

**Materials and Methods**

**Plant material**- TR flowers were collected in 2011 from Corvallis, Oregon. The flowers were solar dried and finely ground for use. The pre-ground Q powder was obtained from Desert King International, San Diego, California. The entire Q plant was used to make the ground material.

**Diet development**- For each of the six treatment groups, the formulation of a tansy ragwort and Quillaja pellet was developed. The six different treatment groups were as follows:

1) 0 weight percent TR, 0 ppm Q powder
2) 2.5 weight percent TR, 0 ppm Q powder
3) 5 weight percent TR, 0 ppm Q powder
4) 0 weight percent TR, 500 ppm Q powder
5) 2.5 weight percent TR, 500 ppm Q powder
6) 5 weight percent TR, 500 ppm Quillaja powder

The pellets were formed using a 10 weight percent corn starch base mixture. The corn starch was mixed with warm water on a hot plate for approximately five minutes. This corn starch solution was then readily mixed with the premixed dry contents until uniform. The dry contents included a ground Harlan Teklad rodent diet (w) 8604 pellets along with the ground TR and/or Q plant material, according to the above treatment groups. This mixture was then processed through a food processor to make pellets.
which were dried in an oven for two days at 100° F. Pellets were stored in plastic bags at room temperature until use.

**Animals** - 36 weanling Wistar rats were purchased from Simonsen Laboratories Inc., Gilroy, California. The rats were individually caged and randomly assigned to one of the six dietary treatment groups. The animals had food and water readily available throughout the entire experiment. Body weight was determined before feeding began and feed intake data was monitored and recorded twice a week. The rats were weighed once a week. Noticeable changes in the animals, such as lethargy, decreased appetite, and changes in behavior were documented within the feeding period. At the end of the 48 day experimental period, the animals were humanely euthanized and necropsied. Liver, heart, and spleen samples were weighed and recorded. Blood samples were also collected. Liver sections were processed for histological examination. All protocols for animals used in this research were performed under veterinary supervision and reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Utah State University, Logan, UT.

**Results and Discussion**

Upon the start of experimental feeding, immediate decreased pellet consumption by the TR treatment groups occurred. The treatment groups that received zero TR in the diet consumed about 20 g per day whereas the groups that received the TR diet consumed anywhere from 10-15 g per day (figure 1). By small scale chemical extraction paired with GC-MS and LC-MS for analysis, 2.19 mg of PA were found per gram of the TR sample. Figure 2 shows the amount of PA’s consumed by each treatment group per day. Besides the lack of weight gain in the rats consuming the TR diets, the rats initially appeared to be otherwise healthy. The rats were weighed once a week and a “final weight” was determined using the
average of the last two weighings (figure 3). Based on the last two weighings, there was no significant difference (2.5% TR p-value= 0.32, 5% TR p-value= 0.56) between the groups that consumed the Q-powder and those that did not. Starting on day 42 of experimental feeding, some of the rats began to appear sickly. One of the rats in the 5% TR and 0 ppm Q powder demonstrated signs of lethargy, jaundice, and stopped eating. Similar signs were observed, just three days after, in another rat in the same treatment group. These rats died on days 44 and 47, respectively. During the last week of experimental feeding, the rats in the 5% TR treatment groups began to show signs of lethargy, decreased appetite, jaundice, and distended abdomens. These same symptoms, yet less dramatic and in fewer rats, were found in the treatment groups receiving the 2.5% TR. All of the rats were humanely euthanized by CO$_2$ gas on day 48 of experimental feeding.

At necropsy, liver, heart, and spleen samples were taken for signs of pyrrolizidine alkaloid toxicosis, such as centrilobular necrosis, megalocytosis, karyomegaly and/or fibrosis, in order to assess the effects of Q saponins on the toxicity of hepatotoxic PA from TR. Liver, heart, and spleen weights were also recorded. The treatment group consuming 2.5% TR and 0 ppm Q powder showed distended abdomens, ascites, grandular livers, and edema. The 5% TR treatment group, without Q powder showed similar, yet more severe, signs as compared to the previous treatment group along with hydrothorax. The groups fed Q powder showed very similar signs of toxicity, along with enlarged spleens, jaundice, and anemia. Spleen hypertrophy is characteristic of PA toxicosis and is a reflection of impaired hematopoesis (Cheeke 2011). The weights of the spleen and liver, as a percent of final body weight, are in figures 4 and 5, respectively. None of the treatment groups showed significant differences in comparing organ weights between equal amounts of TR, with and without Q powder. Liver histology did not show a significant difference between Q powder treated rats and those that did not consume the Q powder.
Figure 1. Comparison of average feed intake (g) per chick per day for each treatment group over the experimental feeding period.

Figure 2. Comparison of the average amount of pyrrolizidine alkaloid (mg) consumed from tansy ragwort per kg body weight per day of the rats for each treatment group.
Figure 3. Comparison of average final body weights (g) of each chick for each treatment group over the experimental feeding period.

Figure 4. Comparison of average spleen weights, as a percentage of the final body weight, of each chick for each treatment group.
Conclusion

This research suggests that 500 ppm of dietary Quillaja does not affect pyrrolizidine alkaloid toxicity from tansy ragwort in rats. Further histological examination is required to fully understand the results, but based on behavioral observations, feed consumption, weight, gross necropsy observations, liver, heart, and spleen weights, there was no difference between adding the Quillaja to the diet compared to not adding it.

Acknowledgements

The authors thank Desert King International, San Diego CA, for the donation of whole plant quillaja powder used in this study.

Figure 5. Comparison of average liver weights, as a percentage of the final body weight, of each chick for each treatment group.
Sources


