Appearance and disappearance of swainsonine in serum and milk of lactating ruminants with nursing young following a single dose exposure to swainsonine (locoweed; Oxytropis sericea)\textsuperscript{1,2}

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ABSTRACT: A series of experiments were conducted to investigate the elimination of swainsonine in the milk of lactating ruminants following a single dose oral exposure to swainsonine (locoweed; Oxytropis sericea) and to assess subsequent subclinical effects on the mothers and their nursing young. In a preliminary experiment, lactating ewes were gavaged with locoweed providing 0.8 mg swainsonine/kg BW (n = 4; BW = 75.8 ± 3.6 kg; lactation = d 45) and lactating cows were offered up to 2.0 mg swainsonine/kg BW free choice (n = 16; BW = 389.6 ± 20.9 kg; lactation = d 90). Serum and milk were collected at h 0 (before treatment), 3, 6, 12, and 24 for ewes, and h 0 (before treatment), 6, 12, 18, and 24 for cows. Swainsonine was highest (P < 0.05) by h 6 in the serum and milk of ewes. Consumption of at least 0.61 mg swainsonine/kg BW induced consistent (\(> 0.025 \mu g/mL\)) appearance of swainsonine in cow serum and milk. In response to the results obtained in the preliminary experiment, a subsequent experiment utilizing lactating ewes (n = 13; BW = 74.8 ± 6.4 kg; lactation = d 30) and cows (n = 13; BW = 460.8 ± 51.9 kg; lactation = d 90) was conducted. Each lactating ruminant was gavaged with a locoweed extract to provide 0 (control), 0.2, or 0.8 mg swainsonine/kg BW and individually penned with her nursing young. Serum and milk from the mothers and serum from the nursing young were collected at h 0 (before treatment), 3, 6, 9, 12, 24 and 48 (an additional sample was obtained at h 72 for ewes and lambs). Serum and milk swainsonine was higher (P < 0.05) in the 0.8 mg treated groups and maximal (P < 0.05) concentrations occurred from h 3 to 6 for ewes and h 6 to 12 h for cows (P < 0.05). Rises in alkaline phosphatase activity indicated subclinical toxicity in the treated ewes (P < 0.05). Following a single dose oral exposure to 0.2 and 0.8 mg swainsonine/kg BW provided by a locoweed extract, swainsonine was detected in the serum and milk of lactating ewes and cows, and rises in serum alkaline phosphatase activity were observed in the ewes. Neither swainsonine nor changes in alkaline phosphatase activity was detected in the serum of the lambs and calves nursing the ewes and cows dosed with swainsonine.

Key Words: Cows, Ewes, Milk, Oxytropis, Swainsona, Lactation

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Introduction

Based on producer testimonials, James and Hartley (1977) investigated the potential of locoweed toxicity to occur in lambs and calves ingesting milk from mothers consuming moderate to high amounts of locoweed. In both species, subclinical and clinical symptoms of toxicity were observed within 7 and 21 d, respectively. Since these studies were conducted prior to the isolation of swainsonine from locoweed (Molyneux and James, 1982), the estimated levels of swainsonine exposure and amount eliminated in the milk were not determined.

Previously, Taylor et al. (2000) suggested that short-term (< 28 d) consumption of 0.2 mg swainsonine/(kg BW·d) or less to be a potential minimum effective level in yearling wethers. This seems to agree with Stegelmeier et al. (1999) who reported minimal changes in some serum constituents (α-mannosidase, aspartate aminotransferase, alkaline phosphatase) and localized (rather than widespread) vacuolization of tissues (pancreas, kidney, thyroid) of wethers consuming less than 0.2 mg swainsonine/(kg BW·d) for 30 d. When considering the findings of James and Hartley (1977), coupled with the normal route of swainsonine elimina-

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treatment), and 6, 12, 18, and 24 h after treatment. Milk and blood sampling were conducted at h 0 (prior to treatment), and 3, 6, 12, 18, and 24 h after treatment. Additional samples were collected at h 72 for the ewes. Blood samples were also obtained from the nursing young at each corresponding sampling hour. Animal use for all experiments was approved and followed the guidelines of the Institutional Animal Care and Use Committee (98-0011, 98-0026).

Materials and Methods

Animals, Treatments, Sampling Intervals

Preliminary Experiment 1. Four lactating ewes (BW = 75.8 ± 3.6 kg; lactation = d 45), given ad libitum access to an alfalfa-based diet, were each placed in an individual pen with her single lamb. Locoweed (Oxytropis sericea; 0.614 mg swainsonine/g of plant matter) intake for each ewe was estimated based on a target swainsonine dosage of 0.8 mg swainsonine/kg BW. Milk and blood samples were obtained at h 0 (prior to treatment), and 3, 6, 12, and 24 h after treatment. A single dose locoweed treatment was delivered by gavage immediately following h 0 of sampling.

Preliminary Experiment 2. Sixteen lactating cows (BW = 389.6 ± 20.9 kg; lactation = d 90), receiving a sudan grass hay diet fed at 1.9% BW and supplemented with 908 g/d of a 46.8% CP supplement, were each placed in an individual pen with her single calf. Based on a maximum intake of 2.0 mg swainsonine/kg BW, a predetermined amount of locoweed (Oxytropis sericea; 0.614 mg swainsonine/g of plant matter) was provided individually and free choice to each cow (calves were denied access); the locoweed was immediately offered after obtaining blood and milk samples at h 0. Cows were allowed 2 h to consume locoweed treatment before removal, and refusals were collected and weighed for estimation of swainsonine intake. Milk and blood sampling were conducted at h 0 (prior to treatment), and 6, 12, 18, and 24 h after treatment.

Experiment 3. In two separate trials, 13 ewes (74.8 ± 6.4 kg; lactation = d 30) with single lambs and 13 cows (460.8 ± 51.9 kg; lactation = d 90) with single calves were individually penned with their nursing young and randomly assigned to one of three treatments: 0 (n = 3; control), 0.2 (n = 5), or 0.8 (n = 5) mg swainsonine/kg BW. Prior to treatment (1 d), a 24-h milk production estimate was conducted for ewes and cows. The estimate was based on the total amount of milk collected (mL) at the end of a 3-h period immediately following complete evacuation (milk let-down induced by 2 mL oxytocin i.v.) of milk from the udder and separation from the nursing young. Swainsonine treatments were delivered by gavage to each animal as a locoweed extract (Oxytropis sericea). For both ewes and cows, blood and milk samples were collected at h 0 (prior to treatment) and 3, 6, 9, 12, 24, and 48 h following treatment. Additional samples were collected at h 72 for the ewes. Blood samples were also obtained from the nursing young at each corresponding sampling hour. Animal use for all experiments was approved and followed the guidelines of the Institutional Animal Care and Use Committee (#98-0011, 98-0026).

Locoweed Extract Preparation. The extract was prepared by boiling ground locoweed (1 mm) in methanol for 24 h. The liquid fraction was filtered and methanol was removed by evaporation. Resulting contents were vigorously mixed 1 to 9 with distilled water and centrifuged at 1500 × g for 30 min to separate the lipid and water soluble fractions. The water soluble fraction was decanted and analyzed for total swainsonine (extract = 5.85 and 9.13 mg swainsonine/mL for ewe and cow gavage, respectively) as described below. The fraction was then mixed 1 to 1 with molasses for treatment delivery (gavage). For the control groups, a 1 to 1 water and molasses solution was prepared.

Sample Collection and Processing. Approximately 7 mL of venous blood (jugular) was collected from ewes, cows, lambs, and calves at the designated sampling times. Ten milliliters of milk was collected from each ewe (first 5 mL per half) and 16 mL from each cow (first 4 mL per quarter) at the designated sampling times. Blood was allowed to clot, then centrifuged at 1500 × g, and serum was decanted into 2-mL storage vials. Samples from all experiments were stored at −20°C for subsequent analyses. Serum samples, milk samples, and methanol extracts of locoweed from all experiments were analyzed for swainsonine using a modified α-mannosidase inhibition assay (lower detection limit = 0.025 μg/mL; Li, 1967) described below.

Swainsonine and Alkaline Phosphatase Analyses. For swainsonine analysis, samples were boiled in a water bath for 30 min and centrifuged for 30 min at 11,900 × g. In triplicate, 20 μL of the supernate was transferred to a 96-well plate (well volume = 320 μL) with 100 μL of citrate buffer (79.2 mM, pH = 4.5) and 20 μL of α-mannosidase enzyme (0.025 U/mL; Sigma Chemical Co., St. Louis, MO), then incubated for 15 min at 37°C. Following incubation, 20 μL of p-nitrophenyl α-D-mannopyranoside (20 mM; Sigma Chemical Co.) was added to each well and then incubated for an additional 90 min. The reaction was stopped and color was developed with 80 μL of borate buffer (200 mM, pH = 9.8) added to each well. Optical density was determined at 405 nm (MRX Microtiter Plate Reader; Dynatech Laboratories Inc., Chantilly, VA). Additionally, serum obtained from the ewes, cows, lambs, and calves in Exp. 3 were analyzed for
Preliminary Experiment 1

was calculated, which included only cows that con-
ated using only cows with detectable swainsonine in
means and standard deviations of the swainsonine
milk swainsonine over the sampling period, arithmetic
sons. To visualize potential changes in cow serum and
were identified using preplanned pairwise compari-
cations and differences between sample hour means
were generated using only cows with detectable swainsonine in
the serum and milk. Additionally, an arithmetic mean
and standard deviation for total swainsonine intake
was calculated, which included only cows that con-
sumed a measurable amount of locoweed.

Experiment 3. All data were analyzed as a completely
randomized design with unequally spaced repeated
measures (spatial power law; Littell et al., 1996) using
mixed models procedure of SAS (8.0). Treatment, sam-
pling hour, and corresponding interaction were in-
cluded in the model. When treatment by sampling hour
interactions were detected, preplanned pairwise com-
parisons were estimated to test for difference between
treatments within sampling hour. To estimate change
in alkaline phosphatase activity and swainsonine con-
centration over the sampling period (h 0 to 72) within
each treatment, preplanned pairwise comparisons
were conducted for each sampling hour vs h 0 for alka-
line phosphatase and initial hour of detection for
swainsonine. Linear, quadratic, and cubic effects of
swainsonine treatment on serum and milk swainson-
ine concentrations were also estimated; when an effect
was detected, solutions for the slope of highest hierar-
chical significance (cubic > quadratic > linear) and best
representing the observed biological effect were gener-
at

to estimate the amount of swainsonine per unit
of BW potentially consumed by the nursing young,
the estimated mean swainsonine content of the milk ((g/
/mL) was multiplied by the 24-h milk production esti-
mate (L/d) and further divided by the BW of the corre-
sponding nursing young.

Results and Discussion

Preliminary Experiment 1

Swainsonine was detected in the serum and milk
of the ewes at all sampling points following initial
swainsonine exposure. Mean serum swainsonine con-
centrations were 0.402, 0.568, 0.227, and 0.140 ± 0.045
μg/mL for h 3, 6, 12, and 24, respectively. All sampling
hours were different (P < 0.02) from the others, and a
maximal serum swainsonine concentration occurred
at h 6 (P < 0.02). Mean milk swainsonine concentra-
tions were 0.127, 0.256, 0.174, and 0.086 ± 0.03 (g/mL
for h 3, 6, 12, and 24, respectively. As with serum,
maximal swainsonine concentrations occurred in the
milk at h 6 (h 6 > h 3 and 24; P < 0.05). Swainsonine
rapidly declined in the serum (h 24 < h 3, 6 and 12; P
< 0.02) and milk (h 24 < h 3, 6, 12; P < 0.06) over the
24-h sampling period after maximal concentrations
were obtained. Although not as pronounced, disap-
ppearance of swainsonine from the milk was similar to
that from serum.

Preliminary Experiment 2

The success of the ewe experiment prompted the
potential use of the locoweed gavage in the preliminary
lactating cow study. However, the large volumes of
slurry to be prepared/dosed, coupled with the number
and disposition of cows available, circumvented the
use of this technique. Therefore, cows were offered a
moderate portion of locoweed, free choice, providing
up to 2 mg swainsonine/kg BW for 2 h. Only 13 of 16
cows consumed a measurable amount of swainsonine
with the arithmetic mean amount consumed during
the 2-h exposure period being 0.744 mg swainsonine/
kg (Table 1). A standard deviation of nearly 60% of
the mean intake and a 4.7-fold difference between the
lowest and highest amount consumed suggest much
variation exists in the level of swainsonine voluntarily
ingested when offered free choice to lactating cows not
having been previously exposed to locoweed. Subse-
quent concentrations of swainsonine were detected in
the serum of 13, 7, 6, and 6 cows for h 6, 12, 18, and
24, respectively (Table 1). Only one cow had detectable
(> 0.025 μg/mL) concentrations of swainsonine in the
serum at h 36 and 48. Swainsonine was also detected
in the milk of 7, 8, 12, 13, and 12 cows at h 6, 12, 18,
30, and 36, respectively; following h 36, swainsonine
was no longer detected in the milk of any cow. Interest-
ingly, swainsonine was not present in the milk at h
24 even though most animals that consumed locoweed
had detectable levels of swainsonine in the milk at the
preceding and following sampling hours. Whether or
not this was an actual event has yet to be determined;
the lower limit of the swainsonine assay describe ear-
lier is 0.025 μg/mL; therefore, swainsonine may have
been present but not at a detectable concentration. At
least 0.61 mg swainsonine/kg BW (provided by loco-
weed free choice) appeared to be the minimal intake
level to induce consistent and detectable concentra-
tions of swainsonine in the serum and milk of cows
acutely exposed to locoweed. Regardless of the varia-
tion in intake, swainsonine was identified in the milk,
detected early (h 6) following exposure, and seemed to
require more time to disappear from the milk than the
serum. The wide range of locoweed intake seems to be
the reason for the variation in number of cows with
detectable concentrations of swainsonine in the serum
and milk; cows consuming more locoweed generally

Statistics and Calculations

Preliminary Experiments 1 and 2. Swainsonine con-
centrations in serum and milk of ewes were subjected
to analysis of variance (SAS 8.0; SAS Institute Inc.,
Cary, NC) and differences between sample hour means
were identified using preplanned pairwise comparisions.
To visualize potential changes in cow serum and
milk swainsonine over the sampling period, arithmetic
means and standard deviations of the swainsonine
concentrations within each sampling hour were gener-
ated using only cows with detectable swainsonine in
the serum and milk. Additionally, an arithmetic mean
and standard deviation for total swainsonine intake
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treatments within sampling hour. To estimate change
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was detected, solutions for the slope of highest hierar-
chical significance (cubic > quadratic > linear) and best
representing the observed biological effect were gener-
at

to estimate the amount of swainsonine per unit
of BW potentially consumed by the nursing young,
the estimated mean swainsonine content of the milk ((g/
/mL) was multiplied by the 24-h milk production esti-
mate (L/d) and further divided by the BW of the corre-
sponding nursing young.
Table 1. Mean (arithmetic) swainsonine intake and concentrations in the serum and milk of lactating cows allowed 2 h access to locoweed (*Oxytropis sericea*), providing up to 2.0 mg swainsonine/kg BW

<table>
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<th>Intake</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean</th>
<th>SD&lt;sup&gt;b&lt;/sup&gt;</th>
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<th>Mean</th>
<th>SD&lt;sup&gt;b&lt;/sup&gt;</th>
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<sup>a</sup>N = number of animals that consumed locoweed (Intake) or number of animals with detectable (> 0.025 μg/mL) levels of swainsonine in the serum or milk.

<sup>b</sup>SD = Standard deviation.

had detectable levels at more sampling hours and more swainsonine present in milk and serum.

**Experiment 3**

Due to some problems in preparing and orally delivering a locoweed slurry as in Exp. 1 and the variation in locoweed intake observed in Exp. 2, alternate methods to administer swainsonine in an inexpensive, rapid, concentrated way were explored. By using methanol to extract large quantities of swainsonine from ground plant matter followed by a series of concentration procedures (e.g., evaporation, centrifugation), an end product was produced that was concentrated and easy to deliver to both ewes and cows. Subsequently, this method was employed for Exp. 3.

**Ewes.** A treatment by sampling hour interaction was detected (*P < 0.001*) for ewe serum and milk swainsonine. From h 3 to 24, swainsonine was detected in ewe serum (Figure 1) and milk (Figure 2) of both swainsonine treated groups. No swainsonine was detected in the serum or milk of the control ewes at any sampling hour and in the serum or milk of the treated ewes beyond h 24 (h 48 and 72). At sampling h 3, 6, 9, and 12, ewes receiving 0.8 mg swainsonine/kg BW had higher (*P < 0.05*) serum swainsonine concentrations than the 0.2 mg treated ewes. Serum swainsonine in ewes dosed with 0.2 mg swainsonine/kg BW remained fairly constant with minimal (*P > 0.40*) fluctuation from initial hour of detection (h 3) to h 24. Maximal (*P < 0.001*) concentrations of serum swainsonine in the 0.8 mg treated ewes were observed at h 3 and 6 (h 3 and 6 > h 9 to 24; *P < 0.05*); following h 6, serum swainsonine rapidly declined (h 6 > h 9; *P < 0.05*) as indicated by the quadratic (*P < 0.001*) disposition of swainsonine over time. The near maximal concentrations of swainsonine occurring at h 3 indicated that much of the swainsonine absorption from the gut had occurred between dosing and h 3. Although describing the pharmacokinetics of swainsonine in lactating animals is beyond the scope of this study, the subsequent disposition of swainsonine in the 0.8 mg treatment group over the sampling period appears to be consistent with the results of Stegelmeier et al. (1995a), in which the half-life of swainsonine was suggested to be 20.3 ± 7.2 h. However, the lack of change in serum swainsonine over time in the ewes treated with 0.2 mg swainsonine/kg BW does not appear to support a $T\frac{1}{2}$ estimate of less than 24 h. Furthermore, no difference (*P = 0.47*) was detected between the 0.2 and 0.8 mg treatment groups at h 24. Taylor et al. (2000) reported that wethers consuming 0.8 or 1.6 mg swainson-
Figure 1. Disposition of swainsonine (SW) in serum of lactating ewes following single dose exposure (gavage) to a locoweed extract (Oxytropis sericea; 5.85 mg SW/mL) at h 0. **Indicates difference ($P < 0.05$) between treatments within hour. ***Indicates difference ($P < 0.05$) between sampling hours within treatment. Quadratic fit and solution were generated using individual responses of experimental units within treatment over time.

ine/kg BW had higher levels of serum swainsonine 24-h post locoweed exposure than wethers exposed to 0.2 and 0.4 mg swainsonine/kg BW. This apparent contrast in the disposition of serum swainsonine between the current experiment and Taylor et al. (2000) could be due to the effect of lactation on the movement of swainsonine away from the serum.

No differences ($P > 0.94$) in estimated 24-h milk yield were detected between the 0 (control), 0.2, or 0.8 mg swainsonine/kg BW treated ewes (1.49, 1.56, and 1.58 ± 0.20 L, respectively). The 24-h mean swainsonine concentration of the milk was lower ($P < 0.003$) in the 0.2 than the 0.8 mg treated ewes (0.134 and 0.222 ± 0.015 μg/mL, respectively). The appearance and disappearance of swainsonine in the milk of the treated ewes was visually similar to the serum (Figure 2). The 0.8 mg swainsonine/kg BW treated ewes had higher ($P < 0.05$) levels of swainsonine in milk at h 3, 6, and 9 than did the lower 0.2 mg treated ewes. Swainsonine in the milk of the 0.8 mg group throughout the first 24 h declined linearly ($P < 0.002$) with maximal swainsonine concentration occurring from h 3 to 6 (h 6 > h

Figure 2. Disposition of swainsonine (SW) in milk of lactating ewes following single dose exposure (gavage) to a locoweed extract (Oxytropis sericea; 5.85 mg SW/mL) at h 0. **Indicates difference ($P < 0.05$) between sampling hours within treatment. Linear fit and solution were generated using individual responses of experimental units within treatments over time.
Exposure of lactating ruminants to swainsonine

Figure 3. Activity of alkaline phosphatase in serum of lactating ewes following single dose exposure (gavage) to a locoweed extract (Oxytropis sericea; 5.85 mg SW/mL) at h 0. **Indicates difference ($P < 0.05$) from control within hour. $\psi$ Indicates difference ($P < 0.05$) from 0.2 mg SW/kg BW treatment within hour. $^x$ Indicates difference ($P < 0.05$) from all other sampling hours within treatment.

9 to 24; $P < 0.05$). The rapid appearance of swainsonine in the milk of both treated groups establishes that the mammary system in lactating ewes is a route readily available and utilized for the elimination of swainsonine and, therefore, could have a major effect on $T_{1/2}$ of swainsonine compared to nonlactating sheep.

A treatment by sampling hour interaction was detected ($P = 0.01$) for serum alkaline phosphatase in the ewes. Increased activity ($P < 0.01$) in serum alkaline phosphatase (Figure 3) was observed in ewes receiving 0.2 mg swainsonine/kg BW at h 24 and at h 6, 12, and 24 for those receiving 0.8 mg. Ewes dosed with 0.8 mg swainsonine had greater ($P < 0.03$) alkaline phosphatase activity than those dosed with 0.2 mg at h 6 and 12, and control ewes at h 6, 12, and 24; this response is similar to the reported alkaline phosphatase activity changes in the serum of wethers (when compared to a control group) 24 h following initial exposure to locoweed (0.8 and 1.6 mg swainsonine/kg BW; Taylor et al., 2000). Alkaline phosphatase activity in the 0.2 mg treated ewes promptly returned ($P > 0.35$) to the activity observed at h 0, and did not differ ($P = 0.59$) from control by h 48.

Cows. A treatment by sampling hour interaction was observed ($P < 0.001$) for cow serum and milk swainsonine. No swainsonine was detected in the serum or milk of control cows. Swainsonine was detected in the serum (Figure 4) of the cows receiving 0.8 mg swainsonine/kg BW at all sampling hours (except h 48) following treatment and was highest from h 9 to 12 (h 9 and 12 > h 3 and 24; $P < 0.05$). In comparison to the lactating ewes, appearance of swainsonine was delayed approximately one sampling interval (3 h). Disappearance of serum swainsonine following maximal levels is similar to that of the ewes and seems to support the $T_{1/2}$ of
Figure 4. Disposition of swainsonine (SW) in serum of lactating cows following single dose exposure (gavage) to a locoweed extract (*Oxytropis sericea*; 9.13 mg SW/mL) at h 0. **Indicates different (*P < 0.05*) between treatments within hour. xyzIndicates difference (*P < 0.05*) between sampling hours within treatment. Cubic fit and solution were generated using individual responses of experimental units within treatments over time.

16.4 ± 3.1 h in nonlactating cows estimated by Stegelmeier et al. (1995a). The cubic response (instead of quadratic as observed with the ewes) obtained in the cows is likely due to the potential capturing of the absorption phase occurring sometime between h 3 and 6. Serum swainsonine was only detected at h 6, 9, and 12 in cows dosed with 0.2 mg swainsonine/kg BW, and as with the lactating ewes, no difference (*P > 0.41*) occurred between sampling hours. Additionally, serum swainsonine in the 0.2 mg treated group was lower (*P < 0.05*) than the values observed in the 0.8 mg treated group at h 6, 9, and 12. The lack of change in the lower treated ewes and cows suggests that the level of swainsonine intake could affect the $T_{1/2}$ of swainsonine in the serum.

No differences (*P > 0.52*) in the estimated 24-h milk yield were detected between 0 (control), 0.2, and 0.8 mg swainsonine/kg BW treated cows (9.06, 9.26, and 7.02 ± 1.98 L, respectively). The 24-h mean swainsonine concentration of the milk was lower (*P < 0.003*) in the 0.2 than the 0.8 mg treatments (0.103 and 0.227 ± 0.021 μg/mL, respectively). In both treated groups,
swainsonine was detected by h 3 in the milk (Figure 5). Peak milk swainsonine concentrations were obtained between h 9 and 12 (h 9 and 12 > h 3, 6, 24; P < 0.05) in cows treated with 0.8 mg swainsonine/kg BW and by h 12 (h 12 > h 3 to 9; P < 0.05) in those treated with 0.2 mg. For the first 24 h, the behavior of swainsonine over the sampling period was cubic (for comparison to the serum, h 48 was not included in regression estimate). Swainsonine was detected in both treated groups at h 48; this effect was expected due to the number of cows in the preliminary experiment that had detectable swainsonine in the milk at h 36 (Table 1). As with the lactating ewes, the similarity in the appearance and disappearance of swainsonine in the serum and milk suggest that swainsonine rapidly moves to the mammary system and the mammary gland serves as a major elimination route.

In contrast to the ewes, serum alkaline phosphatase activities of the cows were not affected (P = 0.53; Table 2) by swainsonine treatment. No data are available describing the acute response of serum alkaline phosphatase activity in the serum of cattle exposed to swainsonine. The choice to use alkaline phosphatase in the cow study was based on the acute response of this serum marker in sheep consuming locoweed (Taylor et al., 2000) and elevated alkaline phosphatase levels observed in cattle by d 7 after commencement of daily locoweed consumption (Bachman et al., 1992).

Lambs and Calves. Body weight did not differ (P > 0.67) between the 0 (control), 0.2, or 0.8 mg swainsonine/kg BW treatment groups for the nursing lambs (14.03, 13.46, and 13.68 ± 1.26 kg, respectively) or calves (101.07, 100.50, and 91.48 ± 7.96 kg, respectively). No swainsonine (P > 0.05) were detected in the serum of lambs or calves nursing mothers in the control or treated groups. For the treated groups, this was unexpected due to the isolation of swainsonine in the milk of both the treated ewes and cows. James and Hartley (1977) observed onset subclinical toxicosis (elevated serum aspartate aminotransferase activity) by d 7 in lambs daily ingesting milk from either cows (via bottle) or ewes consuming 454 and 227 g of locoweed per day, respectively. Moreover, calves nursing the same cows daily also developed subclinical symptoms of toxicity by d 7. As mention earlier, the lower detection limit of the swainsonine assay used for this study is 0.025 μg/mL. Although it is obvious that the nursing young did ingest swainsonine by consuming milk (Figures 2 and 5) from their mothers, further dilution of the swainsonine by the blood and tissues of the lamb or calf coupled with the repeated exposure over the 24-h period apparently reduced swainsonine concentrations to less than the 0.025 μg/mL detection limit. In support of this, the estimated 24-h swainsonine intake by the nursing young of the 0.2 and 0.8 mg swainsonine/kg BW treatments was 0.016 and 0.026 ± 0.002 mg/kg BW for the lambs and 0.009 and 0.017 ± 0.002 mg/kg BW for the calves, respectively. This estimated intake by the nursing young is 12- to 48-fold lower than consumed by the corresponding mothers. Furthermore, the lambs and calves exposure to swainsonine via the milk occurred multiple times over a 24-h period unlike the single dose administered to the lactating ewes and cows.

In addition to the lack of swainsonine being detected, no changes in alkaline phosphatase activities (P > 0.05) were detected in the serum of lambs or calves nursing mothers in the treated or control groups (Table 2). The equivalent locoweed (based on 0.614 mg swainsonine/g plant matter) intake for the lactating animals treated with 0.8 mg swainsonine/kg BW was approximately 98.6 ± 11.4 and 58.3 ± 36.7 g for the ewes and cows, respectively. When compared to the study of James and Hartley (1977), the ewes and cows in the current study consumed an estimated 56% less and 22% more locoweed, respectively. Due to the high variability in swainsonine content of locoweeds (0.15 to 1.2 mg/g plant matter), caution must be exercised in

### Table 2. Serum alkaline phosphatase activitya of ewes and cows and their nursing young (lambs and calves), following a single dose exposure (gavage) of the ewes and cows to 0 (control), 0.2, or 0.8 mg swainsonine/kg BW provided by a locoweed extract (Oxytropis sericea)

<table>
<thead>
<tr>
<th>Item</th>
<th>0 (control)</th>
<th>0.2</th>
<th>0.8</th>
<th>P-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewesbc</td>
<td>67.75 (51.56)</td>
<td>116.73 (39.33)</td>
<td>164.43 (39.33)</td>
<td>P = 0.36</td>
</tr>
<tr>
<td>Lambsb</td>
<td>230.10 (46.06)</td>
<td>259.76 (35.68)</td>
<td>212.78 (35.68)</td>
<td>P = 0.66</td>
</tr>
<tr>
<td>Cowsc</td>
<td>38.63 (16.59)</td>
<td>22.78 (14.36)</td>
<td>19.12 (12.85)</td>
<td>P = 0.65</td>
</tr>
<tr>
<td>Calvesb</td>
<td>82.30 (14.01)</td>
<td>77.93 (10.91)</td>
<td>69.38 (10.91)</td>
<td>P = 0.75</td>
</tr>
</tbody>
</table>

aData main means (± SE) were generated from a repeated measure sampling (h 0, 3, 6, 9, 12, 24, and 48 for mothers and nursing young with an additional sample obtained at h 72 for ewes and lambs only) of the blood (jugular) before (h 0) and after gavage.

bA treatment by sampling hour interaction (P < 0.05) was detected for the ewes only; therefore, treatment differences existing within hour are presented in Figure 5.

cFollowing swainsonine treatment, ewes and cows were individually penned with their nursing young allowing constant individual access of nursing young to milk.
assuming that lower intakes (compared to James and Hartley, 1977) were a reason for the lack of effect on alkaline phosphatase or no swainsonine being identified in the lambs. The lack of noticeable rises in serum alkaline phosphatase activities in the calves and lambs nursing treated mothers would suggest that either no significant levels of swainsonine were transferred to the nursing young to induce measurable subclinical or clinical symptoms of acute swainsonine toxicity or, as with the cows, serum alkaline phosphatase may not be an effective marker for subclinical swainsonine toxicity in young animals. Perhaps other markers, such as serum α-mannosidase (Stegelmeier et al., 1995a, 1999) or serum iron (Taylor et al., 2000), could prove to be better markers of acute swainsonine exposure in young nursing ruminants.

Implications

Swainsonine appeared in the serum and milk of lactating ewes and cows in a dose-dependent fashion following a single dose oral exposure; thus, confirming the mammary system to be a route of swainsonine elimination and source of swainsonine exposure to the nursing young. However, detectable levels of swainsonine and/or subclinical toxicity were not observed in the serum of nursing lambs or calves. Therefore, in order to transfer a sufficient amount of swainsonine to lambs and calves via the milk to subsequently induce detectable levels of swainsonine (>0.025 μg/mL) and/or subclinical toxicity in the serum, a single oral dose of swainsonine (locoweed extract) greater than 0.8 mg/kg BW to the lactating mothers must occur. Based on this and results of others, the greater risk of swainsonine toxicity seems to be when nursing ruminants repeatedly (daily; subacute exposure) select a diet containing locoweed in addition to ingesting milk contaminated with swainsonine.

Literature Cited


