Maternal ingestion of locoweed
III. Effects on lamb behaviour at birth

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Abstract

Locoweed (Oxytropis sericea) is a toxic plant commonly found on rangelands in the western United States. The locoweed toxin is an indolizidine alkaloid, swainsonine that causes neurological and systemic damage. The objective of this study was to determine if feeding of locoweed to pregnant ewes during days 100–130 of gestation would affect lamb behaviour shortly after birth. Twenty yearling nulliparous Columbia–Targhee ewes were divided into two treatment groups: loco ewes fed a 10% locoweed (O. sericea) pellet; control ewes were fed alfalfa pellets. Locoweed pellets contained 0.01 mg/g swainsonine with a mean consumption of 0.32 mg swainsonine (kg−1 day−1 ewe−1). Lambs from the two groups did not differ in birth weight (P = 0.35), primarily because four control ewes had live twins whereas loco ewes had only live singles. Loco lambs were slower (P < 0.01) in latency from birth to first successful standing event compared to control lambs (65 min versus 22 min, respectively). None of the loco lambs successfully nursed their dams up to 120 min post partum, whereas control lambs all sucked successfully at 35 min (P < 0.01). Loco lambs had slower (P < 0.01) times (x̄ = 164 s versus 11 s, respectively) through a progressive maze on days 2, 4, and 6 compared to control lambs. Loco lambs were also much slower (P < 0.01) to reach their dams during a barrier test at 12 h post partum compared to controls (193 s versus 42 s, respectively). Control lambs discriminated their dams at 12 h post partum whereas most loco lambs did not. In this same test, control lambs spent a higher percentage (P = 0.03) of their time in close proximity to their dam (x̄ = 53%) compared to loco lambs (x̄ = 19%). Lambs exposed to locoweed in utero for 30 days were intoxicated at birth. Lambs born to loco ewes showed impairments in their behaviour and appeared to demonstrate a weakened maternal infant bonding. Loco lambs would not have survived at birth without human intervention to assist in nursing.

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The research protocol was approved by the Utah State University Institutional Animal Care and Use Committee.

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1. Introduction

Locoweeds (Oxytropis and Astragalus spp.) are toxic plants commonly found on rangelands in the western United States. The Astragalus and Oxytropis genera are closely related, and grow together on many of the same rangelands. Both are called locoweeds because they contain the same toxin, the indolizidine alkaloid, swainsonine (Molyneux and James, 1982). Ingestion of either Astragalus or Oxytropis causes the same clinical signs (Stegelmeier et al., 1995). Other swainsonine-containing plants (including Ipomoea and Swainsona) are found in South America, Mongolia, China, and Australia. Swainsonine inhibits lysosomal alpha-mannosidase (Molyneux et al., 1985) and Golgi mannosidase II, which interferes with the processing of N-linked glycoproteins by blocking the formation of complex oligosaccharides (Colegate et al., 1985; Tulsiani et al., 1989). Cytoplasmic vacuolar degeneration occurs in the neurons of the central nervous system and in parenchymatous organs (Van Kampen and James, 1969; Stegelmeier et al., 1994).

Animals ingesting locoweed for more than 2 weeks show a loss of motor skills, diminished proprioception, emaciation, and reproductive disorders (Van Kampen and James, 1969). Feeding locoweed during gestation results in birth defects, low birth weights, abortion, or neonatal death (Panter and James, 1985). Previous work demonstrated that locoweed ingestion during gestation negatively influenced ewe behaviour at birth (Pfister et al., submitted for publication). Ewe behaviour at lambing can have a negative impact on lamb sucking behaviour (Dwyer and Lawrence, 1998, 1999); conversely inappropriate lamb behaviour (e.g., poor teat seaking) may limit survival as well (O’Connor and Lawrence, 1992). Lamb survival is greatly impacted by sucking behaviour at birth (Hart, 1985). Further, lamb sucking behaviour immediately following birth is important because ingestion of colostrum facilitates filial bonding (Goursaud and Nowak, 1999). Thus, the objectives of this study were to: (1) determine if lambs from ewes fed locoweed during gestation between days 100 and 130 were behaviourally different at birth from control lambs; (2) determine if locoweed impaired the ability of lambs to discriminate their own dam shortly after birth; (3) determine if the lambs’ ability to traverse a progressive maze was altered during the first week after birth.

2. Materials and methods

2.1. Survivability–viability

Twenty randomly selected yearling nulliparous Columbia–Targhee ewes (63 ± 3 kg) had estrus induced during fall using vaginal pessaries, and were hand-mated twice to two fertile rams. All ewes were bred to lamb within a 10–14 day period. Columbia–Targhee crossbred ewes are a common breed in the Western U.S. with good mothering ability (Simmons and Ekarius, 2000).

Locoweed (Oxytropis sericea) was collected during flowering from the Raft River Mountains of northern Utah. It was brought to the Poisonous Plant Research Laboratory in Logan, Utah, air dried, ground through a 2-mm screen, and stored in plastic bags at 15°C until use. Storage does not affect the toxin concentration (R.J. Molyneux, personal communication). Locoweed pellets were commercially made to contain 10% locoweed, 85% alfalfa, and 5% molasses (i.e., binder) by weight. Alfalfa hay pellets (100%) from the same manufacturer served as the basal diet for the control ewes. The two pellets were similar in crude protein concentration (control = 17.1% and locoweed = 16.1%) and dry matter (control = 93.0% and locoweed = 94.1%). Swainsonine analysis of pellets was done using the method of Molyneux et al. (1991) and is reported on dry weight basis.

Ten randomly selected ewes were fed the 10% locoweed (O. sericea) pellet during gestation between day 100 and 130; 10 control ewes only received alfalfa pellets. All 20 ewes were group-fed and housed together except from 100 to 130 days into gestation when individual animals from both treatments were housed and fed individually. Fresh water and mineral block were available at all times. During the 30-day locoweed-feeding period, ewes were offered pellets ad libitum each morning following the removal and weighing of orts. Ewes were monitored hourly starting at 140 days for onset of parturition. Each ewe was monitored from the onset of parturition until first suckling or 2 h after parturition (whichever came first) for:
time of labor, complications during birth, number of lambs born, sex, body length, weight, physical deformities, heart rate, ewe mothering ability, time to stand, and latency to nurse. In the case of multiple births, the weakest lamb from each set of twins was removed at birth.

Lamb viability was assessed after a physical examination. A pilot study in 10 lambs was conducted prior to the trials to evaluate and refine the assessment protocol at birth; all evaluations were conducted by the same observer. Prior to handling, the ewe was allowed to sniff and lick the lamb for 5 min to facilitate bonding before the lamb was removed (Smith et al., 1966; Klopfer, 1971). The assessment was based on five factors weighted 0–2 points each (i.e., Apgar score), similar to the scoring given to human infants (Apgar and Beck, 1972): (1) heart rate, (2) breathing, (3) activity, (4) appearance, and (5) sucking ability. Heart rate was measured using a heart rate monitor. Respiratory breaths were counted to measure breathing. Activity and appearance were subjectively scored based on the efforts of the lamb to struggle against mild restraint, and attentiveness of each lamb to surrounding stimuli, respectively. After a 10 min assessment, the lamb was returned to its dam. The score for sucking was determined later after the lamb had been returned to the mother; lambs nursing without assistance within 2 h post partum were given a score of 2. If a lamb did not nurse within the 2 h period, it was physically attached to the ewe’s nipple, and that individual was scored 1 if it then nursed. Total viability scores were categorized as follows: 0–3 probable death, 4–6 survive with intervention, and 7–10 strong healthy lamb with no intervention required.

2.2. Discrimination of alien versus own mother

This and all subsequent evaluations were conducted indoors in a large climate-controlled (16°C) barn. Lambs were tested for their ability to discriminate their dams from an alien ewe at 12 and 36 h (±30 min) post parturition. A modified maze was constructed using metal panels according to Nowak et al. (1987), with the addition of a visual barrier (i.e., neoprene sheet stretched across a panel) between the ewes and a 0.5 m physical/visual barrier (i.e., plywood sheet) placed between zones 3 and 4 (Fig. 1). Visual cues may be important to the neonate to discriminate between its mother and an alien ewe (Shillito, 1975; Alexander, 1977; Alexander and Shillito, 1978; Arnold et al., 1975), thus, an initial incorrect choice forced lambs to find their dam without visual contact. Each lamb was held in the start box facing the ewes until both the mother and the alien ewe bleated, typically only a few seconds, then the lamb was released for a 5 min test. Lambs could contact the ewe through the wire mesh panels but could not nurse. The observer was behind a nearby observation panel. A lamb was considered to have been attracted to ewes and to have made a choice if it spent ≥3 min in zones 3 and 4 combined. Further, a lamb made a correct choice if it spent the majority of this time in zone 3 or 4 adjacent to its dam. Ewes were randomly placed at 12 h, and at 36 h (i.e., second test) the position of the dam was alternated with the alien ewe. The alien ewe was chosen based on its expected parturition date deemed to be closest to the
ewe/lamb pair being evaluated; the same alien ewe was used for both tests. Variables recorded were time spent in each zone, time to reach mother, and choice (initial correct or incorrect).

2.3. Barrier test

At 12 h post partum lambs were placed behind a semi-circular physical barrier 2.5 m from their dam (Fig. 2). The dam was restricted within a 1.25 m² physical barrier. Both the lamb and ewe barriers were made of wire panels with 15 cm × 15 cm wire mesh. Lambs were not restrained in any way, and could move in any direction behind or around the barrier. The lamb was released inside the semi-circle facing its dam, and the amount of time the lamb took to reach its dam was recorded with a stopwatch by an observer hidden behind an observation panel.

2.4. Progressive maze

Lambs were given progressively more difficult physical and visual barriers to negotiate on days 2, 4, and 6 post partum. On day 2, the lamb was placed behind a single 1 m × 0.5 m physical and visual barrier located 1 m from its dam. The lamb was briefly held behind the first barrier with its head towards the dam, then released for the evaluation. The ewe was restricted behind a 2 m × 2 m wide cage with one side facing the maze. To successfully complete the test, the lamb had to navigate one or more barriers to reach its dam. Each lamb was given three consecutive opportunities to reach its dam, and the mean time among the three trials was used. On day 4, the same barrier was used as on day 2, with an additional barrier added 0.5 m behind the first on the opposite side of the alleyway. On day 6, the same barriers were used as on day 4, with an additional barrier placed 0.5 m behind the second on the opposite side of the alleyway (Fig. 3). A maximum of 5 min was allowed for each trial.

Fig. 2. Schematic diagram of a semi-circular barrier used to test lamb mobility and propensity to rejoin its dam. At 12 h post partum, lambs were placed behind a semi-circular wire mesh barrier 2.5 m from their dam; dams were held in a 1.25 m² wire-mesh pen. Lambs were not restrained in any way behind the barrier once the test began.

Fig. 3. Schematic diagram of a maze used to test lambs’ ability to negotiate a progressively more challenging maze to reach its dam on days 2, 4, and 6 post partum. Additional partitions as shown were added for the 4- and 6-day tests, respectively.
2.5. Statistical analysis

Student’s t-test was used to test ($P = 0.05$) for differences in birth weights between the two treatment groups. The Wilcoxon rank sum test was used to test for differences in birth vigor between the treatments. The Lifetest procedure of SAS (1999) was used to test for differences in lamb survival and for differences between treatments in time to stand and suck. In the case of the sucking data, the endpoint of interest was time to suck. Because some lambs did not suck without assistance, those observations in which the lambs did not suck were censored to compare nursing latency between the two groups. Fisher’s exact test (two-tailed) was used to compare treatment groups for the initial correct choice of lambs for either their dam or an alien ewe during each test. The percentage of total time spent by lambs with their dam was transformed using arcsine, and analyzed using the mixed model procedure of SAS (1999) with repeated measures on subjects over the 2 test days. Non-transformed means are presented below. For the progressive maze, a mixed model was used, with lambs as random factors nested within treatments, with repeated measures over days.

3. Results

Loco ewes ate 2022 g day$^{-1}$ (S.E. = 39) of locoweed pellets between 100 and 130 days of gestation. Pellets contained 0.01 mg/g swainsonine (DMB), thus ewes consumed 0.32 mg swainsonine (kg$^{-1}$ day$^{-1}$). Two ewes in the loco group gave birth to dead lambs (one single and one set of twins) on day 134 and 138 during gestation, respectively. Lambs from the two groups did not differ ($P = 0.35$) in birth weight ($\bar{x} = 4.89 \pm 0.23$ kg), primarily because four sets of twins were born to control ewes, whereas no live twins were born to loco ewes. Single lambs had higher ($P < 0.01$) birth weights than did twin lambs ($5.6 \pm 0.19$ kg versus $4.2 \pm 0.2$ kg, respectively).

Lambs that were not carried to term (i.e., aborted) showed pathological lesions of locoweed intoxication, with cytoplasmic foamy vacuolation of hepatocytes, convoluted tubule cells of the kidney, and follicular cells of the thyroid.

Greater vigor ($P = 0.01$) at birth was observed in control lambs compared to loco lambs (Table 1). Lambs from the two treatments did not differ (Wilcoxon test $P = 1.0$) in time from birth to first attempt to stand ($\bar{x} = 12.2 \pm 1.7$ min). Loco lambs had a longer latency ($P < 0.01$) from birth to first successful standing event compared to control lambs ($65 \pm 20$ min versus $22 \pm 4$ min, respectively). None of the loco lambs successfully nursed their dams up to 120 min post partum, whereas control lambs all sucked within $35 \pm 5$ min ($P < 0.01$).

Loco lambs had markedly slower mean times through the maze ($P = 0.0009$) on days 2, 4, and 6 compared to control lambs (Fig. 4). Loco lambs had a mean time of $164 \pm 27$ s compared to $11 \pm 2$ s for controls. There was no day × treatment interaction ($P = 0.63$) nor day effect ($P = 0.55$). Loco lambs were also much slower ($P = 0.002$) to reach their dams during the barrier test at 12 h post partum compared to control lambs ($193 \pm 38$ s versus $42 \pm 13$ s, respectively).

Table 1

<table>
<thead>
<tr>
<th>Assessment of lamb vigor shortly after birth determined on five factors: heart rate, respiration, activity, appearance, and sucking ability$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Locoweed</td>
</tr>
<tr>
<td>Control</td>
</tr>
</tbody>
</table>

$^a$ All five factors were summed to determine a total score for lamb vigor. Each factor was scored from 0 to 2 by the same observer, similar to the Apgar score given to human infants. Heart rate was measured using a heart rate monitor; heart rates (HR ± S.E.) did not differ between treatments ($P > 0.28$) and the mean HR score for each treatment was 1.8. Respiratory breaths were counted to measure breathing, and subjectively assigned a score based on evenness of breathing. Activity and appearance were subjectively scored based on the efforts of the lamb to struggle against mild restraint, and attentiveness of each lamb to surrounding stimuli, respectively. The score for sucking was determined after the lamb had been returned to the mother. Means for total score were $9.3 \pm 0.28$ for control lambs and $6.3 \pm 0.27$ for loco lambs ($P = 0.01$).
Table 2

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hours after parturition</th>
<th>Zones 1 + 2</th>
<th>Zones 3 + 4%</th>
<th>Time spent in dam’s zone (%)</th>
<th>Choiceb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Locoweed</td>
<td>12</td>
<td>199 ± 35a</td>
<td>100 ± 34a</td>
<td>39 ± 24a</td>
<td>5/8</td>
</tr>
<tr>
<td>Control</td>
<td>12</td>
<td>59 ± 13b</td>
<td>218 ± 32b</td>
<td>77 ± 8b</td>
<td>1/10</td>
</tr>
<tr>
<td>Locoweed</td>
<td>36</td>
<td>184 ± 45a</td>
<td>115 ± 42a</td>
<td>91 ± 7a</td>
<td>5/8</td>
</tr>
<tr>
<td>Control</td>
<td>36</td>
<td>88 ± 29b</td>
<td>212 ± 30a</td>
<td>88 ± 7a</td>
<td>1/10</td>
</tr>
</tbody>
</table>

a Zones 1 and 2 were triangular-shaped left and right side lanes, respectively (not a physical barrier), originating from a small start box that lambs used before approaching either their dam or an alien ewe. Zones 3 and 4 were each 0.5 m × 2.5 m spaces directly in front of either the dam or an alien ewe. Different letters in the same column (a and b) for either the 12 or 36 h test for Locoweed or control lambs differ (P < 0.05).

b Choice denotes either no choice of either the dam or an alien ewe (i.e., did not spend sufficient time in zones 3 and 4 to determine choice), or an incorrect choice (alien ewe) during 5-min discrimination tests at 12 and 36 h post partum. Different letters in the same column for either the 12 or 36 h test for Locoweed or Control lambs differ (P < 0.05).

Loco lambs performed poorly in discriminating their own dam from an alien ewe (Table 2). Most loco lambs (63%) did not enter zone 3 or 4 (i.e., nearest dam or alien ewe) and thus were considered to have not made a choice during the 12 h test; only one control lamb made no choice. Of those lambs that did make a choice to go to their dam or an alien ewe, control lambs made fewer incorrect choices among alien ewes at 12 h post partum (11%; P = 0.04) compared to loco lambs (67%). At 36 h post partum, controls (78%) did not differ from loco lambs (67%) in correct choice for their dam (P = 0.35). Control lambs spent a higher (P = 0.03) percentage (\( \bar{x} = 53 \pm 8\% \)) of the trial time in the zone (i.e., 3 or 4) next to their dam compared to loco lambs (\( \bar{x} = 19 \pm 8\% \)). There were no age effects (i.e., 12 h versus 36 h test) for amount of time lambs spent near their dam (P = 0.74), nor was there an age × treatment interaction (P = 0.25).

4. Discussion

A sheep’s central nervous system development peaks in mid-to-late gestation (Brooks et al., 1992; Rees et al., 1998), and is affected by maternal swainsonine ingestion (James, 1971). Aborted lambs showed typical cellular vacuolization and other lesions of locoweed poisoning (Stegelmeier et al., 1995). Previous work has also shown that cytoplasmic vacuolization occurred in lambs whose dams were fed locoweed from 100 to 120 days of gestation (James, 1971). In contrast, sheep fetuses exposed to locoweed before 100 days of gestation had no lesions (James, 1971, 1976). Pfister et al. (submitted for publication) gave 10% locoweed pellets containing 0.01% swainsonine to pregnant sheep, causing abortions in 15% of the ewes. Similarly, in this study the abortion rate was 20%, indicating the fetotoxic effects of locoweed feeding in pregnant ewes.

This study provides further evidence that lambs exposed to locoweed in utero for 30 days are intoxicated at birth, and generally these lambs will be unable to survive without human intervention (Pfister et al., submitted for publication). Previous studies have
shown that lambs born to ewes fed locoweed may be weak and underweight at birth (James, 1971, 1972, 1976; James et al., 1969). Nevertheless, little was known about a lamb’s ability to survive or how they compare to “normal” lambs. Human infants are evaluated immediately after birth and at 5 min post partum using the APGAR test. This score has been used to predict infant survival and possible neurological damage at birth (Apgar and Beck, 1972). Lambs exposed to locoweed in utero showed substantial deficits in most measures following birth using a modified APGAR-type test.

Reduced birth weight has a negative effect on neonatal lamb behaviour (Dwyer et al., 2003). Intoxicated lambs that were born alive did not differ from controls in birth weight, probably because four control ewes had twin lambs, and loco ewes had all single lambs. Twin lambs typically weigh less than do singles (Dwyer and Lawrence, 1998). Nonetheless, these locoweed-exposed ewes produced lambs that differed from control lambs in almost all measures of vigor and behaviour. Intoxicated lambs born to locoweed-fed ewes were slower to stand after birth, and were unable to suck without assistance. These results are similar to those of Dwyer (2003) in which lambs born to primiparous ewes were slower to stand and suck compared to lambs born to more experienced ewes. Lambs that are slower to stand and suck are at greater risk of not surviving (Dwyer, 2003).

Pfister et al. (submitted for publication) determined that locoweed feeding during gestation disrupted maternal/infant bonding partially due to aberrant ewe behaviours at birth. In the present study focusing on lamb behaviour, aberrant lamb behaviours appeared to weaken maternal infant bonding. The lack of initial suckling may have disrupted bonding because suckling helps establish the initial bond between lamb and dam (Nowak et al., 1997). Initial lamb activity at birth has been related to lamb survival, and the latency to stand and suck is critical to lamb survival (Dwyer et al., 2001). Dwyer and Lawrence (1999) reported that lamb behaviour is largely independent of the dam shortly after birth, and offspring behaviour had no influence on onset of bonding behaviour of ewes. Their results were with normal lambs, and may not apply to intoxicated lambs that are too feeble to stand and suck normally. Dwyer and Lawrence (1999) also noted that the strength of the ewe–lamb bond was influenced by lamb suckling behaviour in conjunction with dam behaviour. Our previous work has shown that a dam’s behaviour at birth may have a negative impact on lamb suckling behaviour. Dwyer and Lawrence (1998, 2000) also found that lamb suckling behaviour was affected by the dam’s behaviour, with a dam’s cooperation aiding neonatal suckling behaviour.

Treated lambs were slower to traverse a maze to reach their dams, slower to reach their mothers around a barrier, and were unable to discriminate their dam from an alien ewe at 12 h post partum. Discrimination of the dam is an important aspect of maternal bonding (Nowak et al., 1987), and intoxicated lambs performed poorly on discrimination tests 12 h post partum. Lambs of different breeds differ in their ability to discriminate their dam from an alien ewe (Nowak et al., 1987), but control Columbia–Targhee lambs in these tests discriminated their mothers with little difficulty at 12 h post partum. This is earlier than noted in other work, as Nowak et al. (1989) found that lambs were attracted to ewes by 18 h post partum, and could discriminate their own mother at 24 h post partum (Nowak et al., 1989; Terrazas et al., 2002). Ewe behaviour is also important in aiding lambs to recognize their dams. Terrazas et al. (2002) reported that 24-h-old lambs relied more on ewe behaviour to select the dam than on individual physical characteristics of the dam. Establishment of a preferential bond of the lamb with its dam is influenced by sucking behaviour at birth (Nowak et al., 1997). The weaker bond between loco lambs and their respective dams may have resulted from physiological changes near birth. In addition, colostrum from early sucking bouts may also facilitate bonding through a nutritional signal to young lambs (Goursaud and Nowak, 1999).

Sight and hearing are both important senses in a lamb’s ability to locate its dam, although sight may be more important to the neonate than hearing (Shillito, 1975; Alexander, 1977). Nowak (1990) found that vocal lambs were better able to discriminate their dams at 12 h post partum. Our observations suggest that intoxicated lambs were not as vocal as control lambs although we did not record vocalization. In addition, although we did not specifically test lambs for sight or hearing deficits, intoxicated lambs appeared to be dull and depressed, and reluctant to move into the test arena during the 12 h test. This reluctance was reduced at 36 h post partum, as intoxicated lambs improved their
ability to move to and discriminate their dam. Further, intoxicated lambs appeared to lack appropriate neonate bonds with their dams because they also spent less time with their dams once contact was made compared to control lambs, even at 36 h after birth.

Ingestion of locoweed by sheep during gestation has economic implications that go beyond effects on the ewe. Pregnant ewes that graze locoweeds are at risk of aborting their fetus, and neurological damage to moderately intoxicated ewes may be permanent (Hartley and James, 1973). Lambs that are born to previously intoxicated ewes are not normal at birth, and these lambs would be unlikely to survive without assistance. We recommend that pregnant ewes not be allowed to graze locoweeds or other swainsonine-containing plants.

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References


