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# Livestock preference for endophyte-infected or endophyte-free Oxytropis sericea, Ipomoea carnea, and Ipomoea asarifolia

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# Livestock preference for endophyte-infected or endophyte-free Oxytropis sericea, Ipomoea carnea, and Ipomoea asarifolia


## Abstract

Fungal endophyte-infected forages have been shown to alter herbivore feeding preferences. The objective of this experiment was to compare the preference of cattle, sheep, and goats for plants containing (E+) and not containing (E-) fungal endophytes using freshly harvested *Oxytropis sericea*, *Ipomoea carnea*, and *Ipomoea asarifolia*. Goats and sheep rejected all forage choices regardless of endophyte status except for grass and alfalfa hay. Endophyte status had no influence on cattle forage preferences. Cattle rejected all *Oxytropis sericea* E+ and E- choices. Cattle discriminated between *Ipomoea* species, preferring *Ipomoea carnea* to *Ipomoea asarifolia* ($P = 0.004$). In all comparisons, *Ipomoea carnea* was selected over *Ipomoea asarifolia*. Cattle did not discriminate between E+ and E- plants of either species ($P > 0.33$). Cattle preferred E+ *Ipomoea carnea* over E- *Ipomoea asarifolia* ($P = 0.03$), E- *Ipomoea carnea* over E- *Ipomoea asarifolia* ($P = 0.003$), E- *Ipomoea carnea* over E+ *Ipomoea asarifolia* ($P = 0.001$), and E+ *Ipomoea carnea* over E+ *Ipomoea asarifolia* ($P = 0.01$). Nutritional composition, including nonstructural carbohydrate concentrations, did not explain cattle preferences, as *Ipomoea asarifolia* contained higher total carbohydrate concentrations than did *Ipomoea carnea*. The presence of ergot and indole diterpene alkaloids in E+ *Ipomoea asarifolia*, or swainsonine in E+ *Oxytropis sericea* and E+ *Ipomoea carnea* did not influence cattle preference because cattle did not discriminate between E- and E+ plants. This study suggests that for these specific toxic plants, endophyte status plays no part in preferences of grazing cattle. For grazing animals, selection by livestock is related to forage scarcity or low nutrient content in the other available forage.



## Keywords

preference, palatability, cattle, sheep, goats, fungal endophytes

## Cover Page Footnote

We thank Kermit Price for his invaluable technical assistance in all aspects of the study.

## Introduction

Food preferences and diet selection of herbivores are highly dynamic, as animals constantly sample forages to assess the nutrient or toxin content (Wang and Provenza 1997, Villalba and Provenza 2009). Fungal endophyte-infected (E+) forages have been shown to alter herbivore feeding preferences, with mammals (Jones *et al*. 2000, Wäli *et al*. 2013), birds (Madej and Clay 1991), and insects (Clay *et al*. 1985, Clay 1987, Crawford *et al*. 2010) typically preferring endophyte-free (E-) forage. Fungal endophyte-mediated plant-herbivore interactions are complex, and may be driven by various alkaloids produced by endophytes (Saikkonen *et al*. 2013). Even though E- forages may be more acceptable to ruminants, livestock producers may prefer to cultivate pastures with endophyte-infected (E+) forages (e.g., tall fescue, *Lolium arundinaceum*), because E+ grasses often have a fitness advantage over E- grasses, exhibiting comparatively greater growth and grazing tolerance than E- grasses (Asay *et al*. 2001).

Locoweeds are *Astragalus* and *Oxytropis* species (Fabaceae family) containing the indolizidine alkaloid, swainsonine (Cook *et al*. 2014). Swainsonine is produced by a fungal endophyte in locoweeds (Braun *et al*. 2003, Cook *et al*. 2014). Further, members of the Convolvulaceae family *Ipomoea carnea* and *Ipomoea asarifolia* may both contain fungal endophytes (Steiner *et al*. 2006, Markert *et al*. 2008, Cook *et al*. 2013). *Ipomoea carnea* contains an endophyte that produces swainsonine (Cook *et al*. 2014), whereas *Ipomoea asarifolia* contains an endophyte that produces ergoline (Kucht *et al*. 2004, Markert *et al*. 2008, Beaulieu *et al*. 2015) and indole-diterpene alkaloids (Lee *et al*. 2017, Gardner *et al*. 2018).

Choice feeding experiments help our understanding of the feeding behavior of animals, and may allow adjustment of management strategies to improve animal performance (Meier *et al*. 2012). The objective of this experiment was to compare the preference of ruminant livestock (i.e., cattle, sheep, and goats) for plants containing (E+) and not containing (E-) fungal endophytes using freshly harvested *Oxytropis sericea*, *Ipomoea carnea*, and *Ipomoea asarifolia*.

## Materials and Methods

**Plant material**. *Oxytropis sericea*: The plants were grown in outdoor research plots at the USDA-ARS facility in Logan, Utah (41° 45’ 24.27 N 111° 48’20.03 W, 1430 m elevation). Endophyte-free and endophyte-positive plants were derived from seeds from the Raft River Mountains of northern Utah where the seed coat was removed (E-) or not removed (E+, Grum *et al*. 2012). Vertical transmission of the endophyte occurs via the seed coat (Ralphs *et al*. 2011). *Ipomoea carnea* and *asarifolia*: Both *Ipomoea* species were grown in a greenhouse under controlled environmental conditions from seed harvested in Brazil. *Ipomoea carnea* and *Ipomoea asarifolia* seeds originated near Patos, Paraíba state, Brazil. Endophyte negative and positive plants were derived from seeds treated or not treated with fungicide, respectively (Cook *et al*. 2013, Lee *et al*. 2017). Endophyte status was verified by the presence or absence of the metabolite(s) produced by the respective endophyte (see below in Forage Analysis: alkaloids).

**Animals, Husbandry, and Treatments.** All procedures were conducted under veterinary supervision, approved by the Utah State University IACUC, and adhered to the ASAB/ABS (2016) guidelines for the use of animals in research. Four Angus yearling heifers (225 ± 20 kg), 4 yearling Suffolk ewes (41 ± 5 kg), and 4 yearling Spanish does (30 ± 4 kg) were adapted for 7 days to 4 × 4 m indoor pens, with wood chips as bedding. Alfalfa hay (*Medicago sativa*) was the basal diet and was fed at 2.5% of body weight in a feed bunk within each pen. Water was provided *ad libitum*.
Training and Initial Exposure to Test Foods

To train the livestock in the procedures, feeders were placed equidistant from one another in the pens, either attached to the side of the pens or placed on the pen floor. For cattle, rubber tubs (150 cm circumference) were placed on the pen floor. For goats and sheep, metal feeders (20 cm x 50 cm) were hung from the sides of the pens. All the animals were trained for 7 days to investigate each feeder by placing 10 to 20 g of rolled barley into each feeder for 10 min, and then exposing each individual animal.

The animals rapidly learned to consume all of the grain in each feeder and then move to the next feeder with minimal delay. After offering grain in the feeders, the animals were fed alfalfa hay at 2.5% of BW. Any hay not eaten by 1700 h was removed for the night. The animals used in these trials were initially naïve to all test foods, therefore they were briefly exposed to reduce novelty. After the initial training to provide animals with experience moving amongst the feeders, all animals were exposed as a group to each of the test foods in the feeders during a 10-min exposure. Further, each animal was given 3 additional individual exposures to each test food when they were exposed for 5 min/day for 3 consecutive days immediately before the trials began.

**Preference Trials.** The preference trials followed the methods of Pfister *et al*. (1996, 2013). In Trial 1 (4 days), cattle, sheep, and goats were fasted overnight and then at 0730 h individual animals were simultaneously offered 200 g (cattle) or 50 g (sheep and goats) of alfalfa hay, endophyte-positive (E+) *Oxytropis sericea*; endophyte-negative (E-) *Oxytropis sericea*, and endophyte-free grass hay (a mix of *Dactylis glomerata,* orchardgrass, and *Bromus inermis*, smooth brome) for 5 min. *Oxytropis sericea* plants were freshly harvested, green material; both hays were cured and dried. Five minutes was considered sufficient for animals to make selections without any influence of negative postingestive consequences. To remove any position bias, each test feed was moved to a new position (i.e., a different feeder numbered 1, 2, 3, and 4) within the pen each day, and the initial trial lasted for 4 days (i.e., 1 rotation around the pen). At the end of this sequence in Trial 2, the animals were offered only E+ and E- two feeders with position changes for 4 consecutive days. In Trial 3 with the *Ipomoea* species, animals were offered E+ and E- *Ipomoea carnea*, and E+ and E- *Ipomoea asarifolia* for a 4-day period in the 4 different tubs or bins to cattle, sheep and goats that had been fasted overnight. The same positional rotation as noted above was used. Additional feed was not added if an individual offering was consumed within the 5-min time limit. Refusals were weighed at the end of the time period. At 0830 h the animals were fed alfalfa hay at 2.5% of body weight for 7 h, and then excess feed was removed. This study deliberately used short-duration trials to exclude potential effects from negative or positive postingestive feedback (De Rosa *et al*. 2002, Villalba and Provenza 2000).

**Forage analysis: nutritional variables.** Subsamples of all feeds were taken on a daily basis throughout the experimental periods; composited; ground to pass a 1-mm screen in a Wiley mill; and analyzed for dry matter (DM), crude protein (CP; N × 6.25; LECO FP-528 Nitrogen Analyzer, LECO Corp., St. Joseph, MI), neutral detergent fiber (NDF; ANKOM Fiber Analyzer system, ANKOM Technology, Macedon, NY), and in vitro true digestibility (IVTD; ANKOM Daisy incubator system, ANKOM Technology, Macedon, NY) with rumen inoculum from a ruminally-cannulated cow consuming alfalfa hay. The NDF procedure was modified by addition of heat-stable amylase (Sigma Chemical, St. Louis, MO). All analyses are reported on a DM basis. Nonstructural carbohydrates (starch: alpha-linked glucose carbohydrate; water soluble carbohydrates: simple sugars, disaccharides, oligosaccharides, fructans, and some polysaccharides) were analyzed by standard methods (Vidal *et al*. 2009; Derias 1961) by a commercial laboratory (Dairyland Laboratories, Inc., Arcadia, WI). Total nonstructural carbohydrates are the sum of starch and water soluble carbohydrates.

**Forage analysis: alkaloids.** *Oxytropis sericea* was analyzed for the indolizidine alkaloid, swainsonine. The *Ipomoea asarifolia* and *Ipomoea carnea* plant material used in this study was analyzed for the presence of swainsonine, ergot alkaloids, and indole diterpene alkaloids. When swainsonine was detected, concentrations were measured using a modification of a previously published procedure (Gardner and Cook, 2011). A measured quantity of dried plant material (25 mg) was placed in a 2 mL screw-cap microcentrifuge tube. The ground plant material was extracted in 2% acetic acid (1.5 mL) for 18 h with agitation. After extraction, the samples were centrifuged, and an aliquot of the extract (0.5 mL) was diluted in 20 mM ammonium acetate (0.5 mL) in a 1 mL auto-sampler vial. Swainsonine was quantitated in samples by LC-MS/MS as previously described (Gardner *et al*. 2001). The detection limit of swainsonine was 0.001% of dry weight using this analytical procedure. Analysis for presence or absence of indole diterpenes and ergot alkaloids was performed according to Lee *et al*. (2017) and Panaccione *et al*. (2012), respectively. However, because of a lack of suitable standards for individual alkaloids, the individual or total concentrations of the ergot and indole diterpene alkaloids were not quantified in the plant material.

**Statistical analysis.** The fixed effects of position, day, treatment (i.e., different test feeds), and the day × treatment interaction on proportion (%) consumed of offered forage was assessed using a generalized linear mixed model with a β distribution, a logit link, and Laplace estimation. Pen (i.e., different animals) was a random effect blocking factor. Pairwise comparisons among treatment means within a given day were adjusted for family-wise Type I error ($\alpha = 0.05$) using the Tukey-Kramer method. The analysis was accomplished using the GLIMMIX procedure in SAS/STAT 12.1 (SAS Institute Inc., Cary, NC) which is suitable for analyzing proportions. Results of plant nutritional and alkaloid analyses were not statistically analyzed, and means and standard errors are given when appropriate. The proportion consumed by cattle for each *Ipomoea* species as well as *Oxytropis* species (E+ and E-) was regressed against arithmetic non-structural carbohydrate concentrations and other nutritional measures using PROC REG for stepwise multiple-regression analysis in SAS/STAT 12.1.

## Results

**Secondary compounds and nutritional content of plant material.** The indolizidine alkaloid swainsonine was detected in the E+ plant material originating from *Oxytropis sericea* and *Ipomoea carnea* (Table 1), but no swainsonine was detected in E- plant material from these two plant species. In addition, swainsonine was not detected in *Ipomoea asarifolia* in either the E+ or E- plant material used in this study. *Ipomoea asarifolia* E+ plant material contained ergot alkaloids and various indole diterpene alkaloids and their isomers (Table 2). Neither indole diterpenes nor ergot alkaloids were detected in the E- plant material from *Ipomoea asarifolia*. Nutrient composition of the forages used in the preference trials is given in Table 3. All test plants were relatively high in crude protein (> 14%) and in vitro digestibility (> 77%). *Ipomoea carnea* was much lower in starch concentration compared to *Ipomoea asarifolia* (Table 3).

Table 1. Swainsonine[1] concentrations (% ± SEM) of the forages used in the preference trials

| Forage | Endophyte + or - | Concentration (%) |
|---|---|---|
| Alfalfa hay | - | ND[2] |
| Grass hay | - | ND |
| *Ipomoea asarifolia* | E- | ND |
| *Ipomoea asarifolia* | E+ | ND |
| *Ipomoea carnea* | E- | ND |
| *Ipomoea carnea* | E+ | 0.026 ± 0.002 |
| *Oxytropis sericea* | E- | ND |
| *Oxytropis sericea* | E+ | 0.058 ± 0.03 |

[1] Ergot and indole diterpene alkaloid concentrations were not quantified because of a lack of suitable standards for most individual compounds, but chemical analyses confirmed that these compounds were present in the endophyte-positive plant material (see Table 2; Gardner *et al*. 2018; Lee *et al*. 2017).
[2]ND=not detected

**Sheep and goat selection.** The sheep and goats refused all of the *Oxytropis sericea* (E+ and E-), and all of the *Ipomoea carnea* and *Ipomoea asarifolia* (data not shown). Sheep consumed most (62%) of the alfalfa hay (daily $\bar{x}$ and SEM: 30.5±2.4 g) and very little (2.6 %) grass hay (1.3±1.2 g), whereas goats selected (55%) alfalfa hay (27.5±4.9 g), and a small amount (14%) of the grass hay (7.2±2.9 g).

**Cattle selection.** In Trial 1 with E+ and E- *Oxytropis sericea*, and alfalfa and grass hay, cattle did not discriminate between E+ and E- *Oxytropis sericea*, and consumption of both was low (Table 4). Cattle selected essentially the same proportions of alfalfa and grass hay (62% of offered); consumption of both alfalfa and grass hay differed ($P < 0.001$) from consumption of both *Oxytropis sericea* choices. In the E+ and E- *Oxytropis sericea* only trial (Trial 2, Table 4), cattle did not discriminate between the E+ and E- forms of *Oxytropis sericea* ($P = 0.41$).

Cattle discriminated between *Ipomoea* species, preferring *Ipomoea carnea* to *Ipomoea asarifolia* ($P = 0.004$, Table 4). In all comparisons, *Ipomoea carnea* was selected over *Ipomoea asarifolia*. Cattle did not discriminate between E+ and E- plants of either species ($P > 0.33$). Cattle preferred E+ *Ipomoea carnea* over E- *Ipomoea asarifolia* ($P = 0.03$), E- *Ipomoea carnea* over E- *Ipomoea asarifolia* ($P = 0.003$), E- *Ipomoea carnea* over E+ *Ipomoea asarifolia* ($P = 0.001$), and E+ *Ipomoea carnea* over E+ *Ipomoea asarifolia* ($P = 0.01$). Cattle consumption of *Ipomoea* species was moderately related to crude protein content ($r = 0.55$, $P < 0.03$), but not related to NDF concentration or in vitro digestibility ($P > 0.22$). Cattle selection was weakly ($P = 0.13$) but negatively ($r = -0.56$) related to starch concentration, but not water soluble carbohydrates or total nonstructural carbohydrates.


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Table 2. Indole diterpene and ergot alkaloids present but not quantified in endophyte-positive (E+) *Ipomoea asarifolia*

| Indole diterpene alkaloids | Ergot alkaloids |
|---|---|
| Terpendole K | Chanoclavine |
| 11-hydroxy-12, 13 epoxyterpendole K | Ergine |
| 6,7 dehydroterpendole A | Lysergic acid α-hydroxyethylamide |
| Terpendole E | Ergonovine |
| Terpendole C | Ergobalansine |
| Paxilline | |
| Paspaline | |
| 13-desoxypaxilline | |
| Paxitriol | |
| Terpendole I | |
| Terpendole A | |
| Terpendole M | |
| Terpendole B | |
| Terpendole D | |
| Terpendole H | |
| Emindole SB | |

Table 3. Nutrient[1] composition (% ± SEM, DMB) of the forages used in the preference trials. Endophyte positive (E+) and endophyte negative (E-) plants were used; alfalfa and grass hay served as controls.

| Forage | Endophyte + or - | NDF (%) | IVTD (%) | Crude Protein (%) | Starch (%) | Water soluble carbohydrates (%) |
|---|---|---|---|---|---|---|
| Alfalfa hay | - | 38.4 | 77.6 | 21.5 | 0.96 | 12.79 |
| Grass hay | - | 54.5 | 79.8 | 13.1 | 0.34 | 17.75 |
| *Ipomoea asarifolia* | E- | 31.8 | 91.6 | 23.2 | 5.55 | 11.84 |
| *Ipomoea asarifolia* | E+ | 27.4 | 89.7 | 21.6 | 5.13 | 11.56 |
| *Ipomoea carnea* | E- | 28.4 | 87.2 | 33.7 | 0.54 | 9.36 |
| *Ipomoea carnea* | E+ | 24.2 | 90.3 | 36.6 | 0.70 | 8.47 |
| *Oxytropis sericea* | E- | 46.2 | 78.7 | 16.3 | 0.21 | 8.84 |
| *Oxytropis sericea* | E+ | 48.2 | 77.4 | 14.3 | 0.30 | 11.20 |

[1]NDF=neutral detergent fiber; IVTD=in vitro true digestibility.

Table 4. Percentage (%) of offered feed ($\bar{x} \pm$ SEM) consumed by cattle in preference trials with endophyte-positive (E+) or endophyte-negative (E-) *Oxytropis sericea* or two E+ and E- *Ipomoea* species (*Ipomoea carnea* and *asarifolia*). Alfalfa and grass hay were controls.

| Trial/Forage offered | Percentage consumed[1] ($\bar{x} \pm$ SEM) |
|---|---|
| *Oxytropis sericea* (Trial 1) | |
| E+ *Oxytropis sericea* | 4.2±2.5 |
| E- *Oxytropis sericea* | 2.3±1.2 |
| Grass hay | 62.8±6.6 |
| Alfalfa hay | 62.4±5.4 |
| *Oxytropis sericea* (Trial 2) | |
| E+ *Oxytropis sericea* | 44.0±12.1 |
| E- *Oxytropis sericea* | 59.6±8.9 |
| *Ipomoea* species (Trial 3) | |
| E+ *Ipomoea carnea* | 28.6±8.8 |
| E- *Ipomoea carnea* | 34.7±10.7 |
| E+ *Ipomoea asarifolia* | 4.1±3.3 |
| E- *Ipomoea asarifolia* | 9.5±5.5 |

[1] There was no difference in cattle preference for E+ or E- *Oxytropis sericea* in Trial 1 (P > 0.25) or Trial 2 (P > 0.40). During Trial 3, cattle discriminated between *Ipomoea* species, preferring *Ipomoea carnea* to *Ipomoea asarifolia* (P = 0.004), but there was no effect due to endophyte (+ or -) status (P > 0.33).

## Discussion

Consistent with previous reports, the *Ipomoea asarifolia* E+ plant material contained ergot alkaloids (Kucht *et al*. 2004, Markert *et al*. 2008, Steiner and Liestner, 2012, Beaulieu *et al*., 2015), and various indole diterpene alkaloids and their isomers (Lee *et al*., 2017). Similarly, the E+ *Oxytropis sericea* and E+ *Ipomoea carnea* plant material contained swainsonine as previously reported (Cook *et al*. 2009, 2013).

Grazing and browsing ruminant livestock consume a wide array of forage species, but typically they develop short- or long-term preferences for some plants while avoiding others

(Provenza 1995, Early and Provenza 1998). Preference has been defined as an animal's discrimination between different feeds on offer and describes what the animal selects when having full option to do so without being constrained by external factors (Parsons *et al*., 1994). These short-term trials were structured to determine if animals' preference differed depending on the presence or absence of fungal endophytes in feeds. Various complex animal and plant factors are likely involved in preferences, including an animal's physiological state (Villalba and Provenza 1999, Villalba *et al*. 2015), and plant concentrations of nutrients and secondary components leading to postingestive feedback that shapes future plant selection (Provenza *et al*. 1992, Provenza *et al*. 1998). All animals used in these trials were in excellent physiological condition, thus this factor had no apparent influence on results. In addition, there were very limited possibilities in these trials for positive or negative postingestive consequences, such as aversive conditioning, because of the limited exposure and the small amount of material offered to, and ingested by, the experimental subjects. Plant physical structure also interacts with the nutritional status of grazing animals, but the influence of forage structure may be most apparent when forage quality is similar among choices (Villalba and Provenza 1999).

One class of plant secondary compounds that may influence herbivore preferences are endophyte-derived compounds in plants (Rodriguez *et al*. 2009). Most plants form symbiotic relationships with endophytic fungi (Christian *et al*. 2016), with various consequences for both plants and potential herbivores. Some endophytes have been reported to deter feeding by mammalian herbivores (Clay 1988, White 1988, Li *et al*., 2004). Various reports have documented the negative effects of endophyte-derived compounds such as ergot alkaloids on feed preferences (Zavos *et al*., 1988, Filipov *et al*., 1998, Panaccione *et al*., 2006, Bhusari *et al*., 2007), although other non-alkaloidal metabolites may also affect herbivory (Rasmussen *et al*. 2009). Sleepy grass (*Achnatherum robustum* (= *Stipa robusta*)) contains an ergot-alkaloid type endophyte (*Epichloë* spp.), and is toxic to horses if consumed for several consecutive days. Typically, once animals experience the psychotropic effects of such grasses, they preferentially avoid those plants, and animals native to regions with sleepy grass do not consume them (Bailey 1903). Tall fescue (*Festuca arundinacea*) is a forage grass planted extensively in the United States. An endophytic fungus in tall fescue, *Epichloë coenophiala* (= *Neotyphodium coenophialum*)), causes chronic toxicity in cattle, and apparently results in cattle preferring to graze other forages when given opportunity for selection (Boland *et al*. 2012, Schaefer *et al*. 2014, Nelson *et al*. 2019). Koh and Hik (2007) suggested that there is a link between endophyte infection in grasses, measured as density of hyphae, and deterrence to herbivores. Rasmussen *et al*. (2007) demonstrated that concentrations of several alkaloids increased with hyphal concentrations of *Neotyphodium lolii* in perennial ryegrass, *Lolium perenne*. Indole-diterpene alkaloids, when present in endophyte-infected ryegrass, are responsible for "ryegrass staggers" (Hovermale and Craig 2001).

Henry *et al*. (2016) reported that ergot and indole diterpene alkaloids reduced sheep intake of perennial ryegrass. Welch *et al*. (2018) conducted behavioral assessments of mice provided chow with various proportions of E+ or E- *Ipomoea asarifolia*, in which the E+ plants contained indole diterpene alkaloids, and reported a dose-dependent decrease in chow consumption as the percentage of E+ plant material in the chow increased. This suggests that feed deterrence in mice was related to the concentrations of endophyte-derived compounds.

**Sheep and Goat selection.** In this study, *Oxytropis sericea* was a novel food to which the animals had been recently exposed; however, even with prior exposure, sheep and goats rejected all of the *Oxytropis sericea*, regardless of endophyte presence or absence. Similarly, sheep and goats rejected both *Ipomoea* species even with pre-trial exposure. In 2 previous field studies with


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sheep grazing in areas with a high density of *Oxytropis sericea*, sheep selected only a few bites of *Oxytropis sericea* (Pfister, unpublished data). Further, Ralphs *et al*. (1991) reported that grazing sheep consumed extremely small quantities of *Oxytropis sericea* (0.08 % of total bites) when grazing a mountain pasture with a high density of endophyte-positive *Oxytropis sericea*. In studies using *Oxytropis sericea* incorporated into alfalfa pellets, typically at *Oxytropis sericea* concentrations of 10 to 20% of the pellet, sheep have generally rejected pellets made with endophyte-positive *Oxytropis sericea* during initial exposure, and a number of days, or even several weeks of periodic fasting and repeated exposure have been necessary to overcome this initial rejection (Pfister *et al*. 1996). However, we speculate that rejection by sheep of *Oxytropis sericea* was not influenced by the presence or absence of endophytes, as sheep have eaten relatively large quantities of pellets made from endophyte-positive (i.e., swainsonine-containing) *Astragalus lentiginosus* (Hartley and James, 1975, James 1976). It is highly unlikely that the alkaloid swainsonine plays a significant role in livestock preference for swainsonine-containing plants or feeds because of the very low concentrations in plant tissue, and the lengthy delay between ingestion and toxicosis (Molyneux and Ralphs 1992). Previous work has shown high variability in sheep acceptance of endophyte-positive feed (Wäli *et al*. 2013), suggesting that animal preference is likely a complex interaction of many factors including endophyte status. Villalba *et al*. (2011) reported almost complete rejection by untreated sheep of endophyte-positive tall fescue (*Festuca arundinacea*). Lisonbee *et al*. (2009) reported that baseline (i.e., pretreatment) intake by sheep given a choice between endophyte-free and endophyte-infected tall fescue varied greatly from day-to-day, with mean consumption of endophyte-positive tall fescue ranging from 20 to 80% of grazing time.

*Ipomoea carnea* is native to tropical America (Austin and Huáman 1996), and is particularly troublesome as a toxic plant in Brazil (Araújo 2008, Tokarnia *et al*. 2012). *Ipomoea carnea* contains the indolizidine alkaloid swainsonine, whereas *Ipomoea asarifolia* contains ergot and indole diterpene alkaloids. Although the toxins differ in these two species, both contain fungal-derived compounds. Some studies suggest that animals that begin to feed on *Ipomoea carnea* eat the plants compulsively regardless of palatability; further, through social facilitation, these animals induce grazing cohorts to consume the plants (Armién *et al*. 2007; Oliveira *et al*. 2009). Both sheep and goats in this study rejected *Ipomoea carnea*. Field (Oliveira *et al*. 2014) and pen (Oliveira *et al*. 2015) studies in Brazil have shown that *Ipomoea carnea* is not highly preferred by sheep or goats, but that grazing animals begin to consume the plant when other forage is scarce; even with scarcity of forage, grazing animals are typically exposed to *Ipomoea carnea* for about 10-14 days before they initiate consumption (Oliveira *et al*. 2014). Likewise, Oliveira *et al*. (2015) reported that experienced or naïve sheep and goats did not prefer *Ipomoea carnea* during preference trials where fresh plant was offered with other feeds or forages.

*Ipomoea asarifolia* is widespread in tropical portions of the Americas, Africa, and south and southeastern Asia (Austin 2005). The plant is especially troublesome for livestock producers in northern and northeastern Brazil, where *Ipomoea asarifolia* poisons buffalo, cattle, sheep, and goats (Araujo *et al*. 2008). Livestock are principally poisoned during the dry season when scarcity of forage apparently drives animals to consume the plant (Barbosa *et al*. 2005, Fabricio *et al*. 2014). Experimental studies using *Ipomoea asarifolia* have characterized the plant as unpalatable (Araujo *et al*. 2008), and at times have had to hand feed plant material because animals did not accept freshly harvested plants (Medeiros *et al*. 2003, Barbosa *et al*. 2005). The goats and sheep in the present study were fasted overnight, but were not in a state of long-term hunger or poor body condition from a lack of forage. These results suggest that the initial rejection of freshly-harvested

*Ipomoea carnea* and *Ipomoea asarifolia* by goats and sheep was not related to endophyte status, but rather a response to relatively unpalatable and novel forages.

**Cattle selection.** *Oxytropis sericea* was not palatable to cattle in these trials when cattle also had access to alfalfa or grass hay. *Oxytropis sericea* is not considered a highly palatable forage for cattle if other forages are available, and if cattle are not accustomed to consume *Oxytropis sericea* (Ralphs *et al*. 1994). Other factors influencing *Oxytropis sericea* consumption by cattle include grazing pressure, progressive intoxication or habituation, and phenological stage of growth. In trials to examine relative preference, Ralphs (1987) showed that cattle initially selected *Oxytropis sericea* only when other forages were depleted. Further, Ralphs *et al*. (1993) reported that cattle with a developed propensity to eat *Oxytropis sericea* (termed “loco-eaters”) consumed modest amounts during spring grazing trials, whereas other cattle with no developed preference for *Oxytropis sericea* (termed “non-eaters”) ate almost no *Oxytropis sericea* during spring grazing. When the *Oxytropis sericea* matured in summer, both groups of cattle consumed little or no *Oxytropis sericea* (Ralphs *et al*. 1993).

Endophyte presence or absence had no effect on cattle preference for either *Oxytropis sericea*, or *Ipomoea carnea* and *Ipomoea asarifolia*. In contrast, Jones *et al*. (2000) reported that cattle preferred endophyte-free robust needlegrass (*Achnatherum robustum*) over endophyte-positive plant material. Lyman *et al*. (2011) found that cattle grazing of endophyte-infected tall fescue plots was cyclic, varying from about 15 to 60% of daily grazing time. Cattle preferred *Ipomoea carnea* over *Ipomoea asarifolia* in paired trials for reasons that are not clear. *Ipomoea carnea* contains the indolizidine alkaloid swainsonine, but swainsonine had no effect on short-term preference by cattle as shown by the lack of differences in cattle preference for E- and E+ *Ipomoea carnea*. Similarly, *Ipomoea asarifolia* contains ergot and indole diterpene alkaloids, which might influence preference by livestock (Henry *et al*. 2016). However, these compounds were present only in the E+ *Ipomoea asarifolia*, and we detected no differences in preference between the E- and E+ *Ipomoea asarifolia*. Jones *et al*. (2000) noted that ergot and loline alkaloids did not play a part in the preference of robust needlegrass by cattle.

The nutritional profile of *Ipomoea asarifolia,* with much higher starch concentrations, suggests that *Ipomoea asarifolia* might be more preferred by cattle compared to *Ipomoea carnea*. Villalba and Provenza (1997) found that lambs preferred straw when the plant material was paired with intraruminal infusions of starch. Mayland *et al*. (2000) reported that cattle grazing preference among tall fescue cultivars was related to the concentrations of total nonstructural carbohydrates ($r^2 = 0.49$, $P < 0.05$); however, other sugar fractions were not related to grazing preference. In related research, Fisher *et al*. (2002) found that ruminant preference for alfalfa hay was positively related to carbohydrate concentrations. Similarly, Burritt *et al*. (2005) found that sheep preference for alfalfa hay was related to increased water soluble carbohydrate concentrations. We found no apparent relationship between carbohydrate fractions and preference by cattle for these 3 plant species.

## Summary

Goats and sheep rejected all forage choices regardless of endophyte status except for grass and alfalfa hay, highlighting their neophobia when exposed to previously novel foods with limited pre-trial exposure (Launchbaugh *et al*. 1997, Provenza *et al*. 1998). Endophyte status had no influence on cattle forage preferences. Cattle rejected all *Oxytropis sericea* E+ and E- choices, and preferred *Ipomoea carnea* to *Ipomoea asarifolia* regardless of endophyte status. Nutritional composition, including nonstructural carbohydrate concentrations, did not explain cattle preferences, as

*Ipomoea asarifolia* contained higher total carbohydrate concentrations than did *Ipomoea carnea*. The presence of ergot and indole diterpene alkaloids in E+ *Ipomoea asarifolia*, or swainsonine in *Oxytropis sericea* or *Ipomoea carnea*, did not influence cattle preference for either *Ipomoea* species or *Oxytropis* sericea because cattle did not discriminate between E- and E+ plants. This study suggests that for these specific toxic plants, endophyte status plays no part in preferences of grazing cattle. Under grazing conditions, these plants are primarily consumed by livestock when other, more desirable forage is scarce or of low nutritional quality.

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