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SWAINSONINE EXCRETION, NUTRIENT DIGESTIBILITY AND NITROGEN RETENTION OF LAMBS FED ALFALFA HAY, LOCOWEED, AND NOVEL FEED ADDITIVES[1]

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ABSTRACT: Novel products are needed that could reduce locoweed toxicity, alleviate impaired performance, and prevent possible death when consumed by livestock. This study evaluated the effect of 3 feed additives on swainsonine intake and excretion, nutrient digestibility, and N retention of 40 wether lambs (39 ± 0.4 kg initial BW). Lambs were blocked by initial BW and assigned to 5 dietary treatments in a randomized complete block design (4 blocks). Treatments were a control diet (86% alfalfa hay and 14% corn-based supplement) fed to lambs at 1.8% of BW (as fed) for 20 d (CON), CON with 20 g/d locoweed replacing alfalfa hay (LOCO), LOCO with 50 g/d of feed additive 1 replacing alfalfa hay (AK1), LOCO with 50 g/d of feed additive 2 replacing alfalfa hay (AK2), and LOCO with 50 g/d of feed additive 3 replacing alfalfa hay (AK3). Lambs were housed individually for 14 d in pens and then for 6 d in metabolism crates for total fecal and urine collections. Statistical analysis used the mixed procedure of SAS with lamb as the experimental unit. Intake, fecal, and urinary swainsonine were greater ($P < 0.05$) for LOCO, AK1, AK2, and AK3 than CON. Intake of swainsonine was lower ($P < 0.05$) for AK3 than LOCO, fecal swainsonine was lower ($P < 0.05$) for AK1 than LOCO, and urinary swainsonine was less ($P < 0.05$) for AK1 and AK2 than LOCO. Treatments did not affect (P 2 0.20) DM intake, fecal DM, or DM digestibility. Nitrogen intake was less ($P < 0.05$) for AK1, AK2, and AK3 than for CON and LOCO, but fecal N and urine N was not affected (P 2 0.11) by treatments. Nitrogen digestibility was not different ($P = 0.26$) among treatments, but N retention was less ($P < 0.05$) for AK1 and AK3 than CON. In summary, lamb consumption of locoweed with the feed additives evaluated in the current study does not significantly affect DM and N digestibility. Decreased fecal and urinary swainsonine in lambs receiving AK1 indicated that it may affect metabolism of swainsonine in sheep.

Key words: nitrogen retention, sheep, swainsonine

INTRODUCTION

Locoweed species (Astragalus and Oxytropis spp.) are poisonous plants responsible for large economic losses estimated at $300 million or more in the livestock industry (Nielsen and James, 1992). Swainsonine, the primary toxin in locoweed (Molyneux and James, 1982), is an inhibitor of a-mannosidase, causes accumulation of oligosaccharides in the lysosomes (Dorling et al., 1980), and alters the processing of glycoproteins in the Golgi (Kang et al., 1993). Many nutrient transporters, such as sodium-dependent glucose transporter-1 and intestinal amino acid regulatory proteins (rBAT and 4F2), are glycoproteins in nature (Wright et al., 1994; Mailliard et al., 1995). Therefore, the effects of swainsonine on synthesis, processing, and transport of glycoproteins may alter gastrointestinal function and nutrient absorption (Pan et al., 1993).

Taylor et al. (2000) reported that lower serum cholesterol, triglycerides, and Fe concentration in sheep consuming swainsonine may be caused by altered nutrient digestion and metabolism. Additionally, Reed et al. (2003) demonstrated that ruminal NH_3 and in situ dry matter digestion were altered when wether lambs were fed locoweed, Reed (2004) reported negative N balance for wether lambs fed 1.6 mg swainsonine per kg of body weight, while control lambs had a positive N balance. Previous research has also demonstrated that swainsonine induces vacuolization of hepatocytes of rats (Novikoff et al., 1985). Preliminary research (unpublished) indicated that supplementation of novel feed products containing a combination bacterial cell walls, yeast, and enzymes minimized the negative effects of swainsonine on rumen epithelial cells and liver weight of sheep fed locoweed. Therefore, the objectives of this experiment were 1) to evaluate the effects of locoweed on nutrient digestion and N balance of lambs, and 2) to evaluate the potential for three novel feed additives (Agri-King Inc., Fulton, IL) to alter swainsonine excretion and minimize the negative effects of locoweed on nutrient digestibility and N balance.

MATERIALS AND METHODS

Experimental procedures were approved by the New Mexico State Institutional Animal Care and Use Committee.

Animals, Design, and Treatments. The experiment was conducted in the Physiology and Nutrition Building at New Mexico State University in Las Cruces, New Mexico. Forty wether lambs (39 ± 0.4 kg initial BW) were equally divided into 4 BW blocks, and within each block were randomly assigned to 1 of 5 dietary treatments in a randomized

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complete block design. The experimental period for each block was 20 d; animals were housed in individual feeding pens from d 1 to 14 for adaptation to dietary treatments, and in metabolism crates from d 15 to 20 for urine and fecal collections. Treatments were a control diet (86% alfalfa hay and 14% corn-based supplement) fed to lambs at 1.8% of BW (as fed) for 20 d (**CON**), CON with 20 g/d locoweed replacing alfalfa hay (**LOCO**), LOCO with 50 g/d of feed additive 1 replacing alfalfa hay (**AK1**), LOCO with 50 g/d of feed additive 2 replacing alfalfa hay (**AK2**), and LOCO with 50 g/d of feed additive 3 replacing alfalfa hay (**AK3**). Locoweed (Astragalus allochrous) was collected in April in southeast New Mexico, allowed to air dry, and passed through a forage chopper (The Western Bear Cat No 5A, by Western Land Roller Co., Hastings, NE) to reduce particle size. The amount of locoweed fed was calculated to supply approximately 2 mg swainsonine per kg of BW daily.

Sample Collections. Feed samples were collected every week after mixing of dietary treatments and stored at -20°C for analysis. Feed refusals were collected daily before the morning feeding, weighed, and then stored at -20°C. Total fecal and urinary outputs were collected daily for 6 days (from d 15 to d 20). Feces was collected into pans attached to metabolism crates and urine was collected via a funnel into 20-L plastic buckets containing 50 mL of HCl (6 M) to trap N and reduce NH_3 loss. Total daily fecal output, and 10% of the daily urine output was frozen at -20°C. At the end of the collection period, feed refusals, and fecal and urine samples were thawed, mixed thoroughly for each lamb, and a representative sample was stored at -20°C to be analyzed later.

Sample Analysis. Diets, refusals and fecal composited samples were weighed, dried for 72 h at 55°C in a forced air oven (Blue M Electric Company, Blue Island, IL), allowed to equilibrate overnight at room temperature, and then weighed to measure moisture loss. Dried samples were ground in a Wiley mill (Model 4, Thomas Scientific, Swedesboro, NJ) to pass a 1-mm screen. All samples of dietary treatments, feed refusals, feces and urine were analyzed for swainsonine concentration using a modified a- mannosidase inhibition assay as described by Taylor and Strickland (2002). Ground samples were analyzed for DM after being placed in an oven (Model 845, Precision Scientific Group, Chicago, IL) at 105°C for 24 h, and for ash after being placed in a muffle furnace at 550°C for 8 h. Samples were analyzed for NDF and ADF using Ankom protocol (Ankom 200 fiber analyzer, Ankom Technology Cooperation, Fairport, NY) by boiling ground samples in neutral detergent solution with a-amylase enzyme for 75 min followed by three times of 5-min washing in hot water for NDF analysis, and in acid detergent solution for 60 min followed by three times of 5-min washing in hot water for ADF analysis. Diets, refusals, fecal, and urinary samples were analyzed for N by measuring N_2 produced upon complete combustion of samples in a thermo-conductivity cell using an N analyzer (Leco, Model FP-528, LECO Corporation, St. Joseph, MI).

Statistical Analysis. The experiment was a randomized complete block design, and all data were analyzed using mixed models (SAS Inst. Inc., Cary, NC) with lamb as the experimental unit. Due to a limited number (10) of the metabolism crates, lambs were equally divided into 4 complete blocks based on BW and date in metabolism crates. The statistical model included treatment, day, and treatment × day interaction as fixed effects, and block was random. When treatment × day interactions were not significant, single degree of freedom contrasts were used to compare CON with the average for all other treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3. Differences among treatments were considered significant when $P < 0.05$.

RESULTS

Intake, fecal, and urinary swainsonine were greater ($P < 0.05$) for treatments containing locoweed than CON (Table 2). Intake of swainsonine was less ($P < 0.05$) for AK3 than LOCO, fecal swainsonine was lower ($P < 0.05$) for AK1 than LOCO, and urinary swainsonine was less ($P < 0.05$) for AK1 and AK2 than LOCO. Treatments did not affect (P 2 0.20) DM intake, fecal DM, DM digested, or DM digestibility. Intake of OM was greater ($P < 0.05$) for AK3 than LOCO, but fecal OM and OM digestibility were not different (P 2 0.62) among dietary treatments. Intake of NDF was less ($P < 0.05$) for AK1 and AK3 than LOCO, and was greater ($P < 0.05$) for AK2 compared with LOCO. No differences (P 2 0.09) were observed among treatments for fecal NDF and NDF digestibility, but grams of NDF digested was less ($P < 0.05$) for AK1 than LOCO. Intake of ADF was greater ($P < 0.05$) for treatments containing locoweed than CON, less ($P < 0.05$) for AK1 than LOCO, and greater ($P < 0.05$) for AK2 than LOCO. Fecal ADF and ADF digestibility were not different ($P = 0.06$) among dietary treatments, but ADF digested was less ($P < 0.05$) for AK1 than LOCO. Nitrogen intake was less ($P < 0.05$) for treatments containing locoweed than CON, and less ($P < 0.05$) for AK1, AK2, and AK3 than LOCO. However, fecal N and urine N was not affected ($P > 0.11$) by treatments. Nitrogen digestibility was not different ($P = 0.26$) among treatments, but grams of N digested was less ($P < 0.05$) for treatments containing locoweed than CON, and less ($P < 0.05$) for AK1, AK2, and AK3 than LOCO. Retained N and N retention was less ($P < 0.05$) for treatments containing locoweed than CON, and lower ($P < 0.05$) for AK1 than LOCO.

DISCUSSION

Effects of Feeding Locoweed. Many nutrient transporters are glycoproteins in nature (Wright et al., 1994; Mailliard et al., 1995), and because swainsonine alters the processing of glycoproteins in the Golgi (Kang et al., 1993), it is possible that locoweed in livestock diets could affect nutrient absorption and metabolism. In the current study, no differences in DM, OM, and NDF intake between lambs fed treatments containing locoweed and CON are likely

Table 1. Dietary treatments fed to lambs

Item	CON	LOCO	AK1	AK2	AK3
Ingredient, g/d					
Alfalfa hay	620	600	550	550	550
Corn grain	95	95	95	95	95
Feed product[1]	0	0	50	50	50
Locoweed[2]	0	20	20	20	20
Molasses	5	5	5	5	5
Nutrient, % DM					
OM	88.8	88.5	88.5	88.5	88.8
NDF	48.4	48.7	47.7	49.9	48.1
ADF	35.8	36.2	35.8	37.6	36.2
CP	18.3	18.4	17.3	17.5	17.7
Swainsonine[3], mg/kg DM	0	149	145	150	123

[1]Novel feed products containing a combination of bacterial cell walls, yeast, and enzymes, with rice hulls as the carrier.
[2]Astragalus allochrous (half moon locoweed).
[3]Analyzed using the modified a-mannosidase inhibition assay as described by Taylor and Strickland (2002).

Table 2. Intake, excretion of swainsonine, and digestibility of DM, OM, NDF and ADF, and N retention of lambs exposed to locoweed toxicity and supplemented with novel feed additives

	Treatments[1]							
Item	CON	LOCO	AK1	AK2	AK3	SEM	*P*-value[2]	Contrast[3]
Swainsonine								
Intake, mg/d	0	98.2	95.9	99.4	81.4	3.93	<0.01	4,7
Feces, mg/d	0	6.35	4.29	6.24	5.18	0.66	<0.01	4,5
Urine, mg/d	0	25.3	19.6	16.4	25.6	1.73	<0.01	4,5,6
DM								
Intake, g/d	660	660	661	660	662	0.74	0.20	NS
Feces, g/d	239	245	254	246	249	6.02	0.52	NS
Digested, g/d	421	415	407	415	413	6.05	0.62	NS
Digestibility, % of intake	63.8	62.9	61.6	62.8	62.4	0.91	0.56	NS
OM								
Intake, g/d	586	584	585	584	588	0.81	<0.01	7
Feces, g/d	204	208	216	209	213	5.73	0.62	NS
Digested, g/d	382	375	369	376	375	5.81	0.64	NS
Digestibility, % of intake	65.2	64.3	63.0	64.3	63.8	0.98	0.64	NS
NDF								
Intake, g/d	319	322	315	330	318	1.12	<0.01	5,6,7
Feces, g/d	138	148	159	148	155	5.86	0.16	NS
Digested, g/d	181	173	156	181	164	6.14	0.03	5
Digestibility, % of intake	56.5	53.9	49.6	55.0	51.5	1.83	0.09	NS
ADF								
Intake, g/d	236	239	236	248	240	0.79	<0.01	4,5,6
Feces, g/d	107	115	126	114	122	4.56	0.06	NS
Digested, g/d	129	124	110	134	118	4.73	0.01	5
Digestibility, % of intake	54.2	51.9	46.7	53.9	48.9	1.91	0.04	NS
N								
Intake, g/d	19.3	19.4	18.3	18.5	18.7	0.05	<0.01	4,5,6,7
Feces, g/d	6.12	6.32	6.11	6.28	6.07	0.15	0.68	NS
Urine, g/d	12.5	13.3	14.6	13.0	14.1	0.56	0.11	NS
Digested, g/d	13.2	13.1	12.2	12.3	12.7	0.14	<0.01	4,5,6,7
Retained, g/d	0.82	-0.22	-2.39	-0.75	-1.47	0.69	0.02	4,5
Digestibility, % of intake	68.3	67.3	66.4	65.9	67.5	0.80	0.26	NS
Retention, % of intake	4.41	-1.76	-13.9	-4.87	-8.34	3.83	0.01	4,5

[1]CON = basal diet of 620 g/d of alfalfa hay and 100 g/d of corn-based feed fed to lambs in equal portions twice daily (0730 and 1930) for 20 d; LOCO = 20 g/d of locoweed replaced alfalfa hay in basal diet; AK1 = 20 g/d of locoweed plus 50 g/d of feed product 1 replaced alfalfa hay in basal diet; AK2 = 20 g/d of locoweed plus 50 g/d of feed product 2 replaced alfalfa hay in basal diet; AK3 = 20 g/d of locoweed plus 50 g/d of feed product 3 replaced alfalfa hay in basal diet. Novel feed products were supplied by Agri-King Inc.
[2]Observed significance level for the type 3 test of treatment fixed effects.
[3]Contrast: 4 = CON vs. average of LOCO, AK1, AK2, and AK3 ($P < 0.05$); 5 = LOCO vs. AK1 ($P < 0.05$); 6 = LOCO vs. AK2 ($P < 0.05$); 7 = LOCO vs. AK3 ($P < 0.05$); NS = all contrasts were not significant ($P > 0.05$).

because the total feed offered (as fed) was limited to 1.8% of BW (DM intake was 1.7% of BW) to encourage complete consumption of the diets and to minimize selective refusal of the supplement containing locoweed and feed product. Our findings are in contrast to Pfister et al., (1996), who observed decreases in intake when sheep were fed alfalfa pellets containing 10% locoweed at 1.6% of BW. Also, Obeidat (2004) reported decreases in DM intake when the diet of limit-fed (1.8% of BW) sheep contained 20% locoweed. Although the contrast that compared the average of the locoweed-containing treatments with the CON showed significant differences for ADF intake, N intake, and N digested, these responses appeared to not be due to the locoweed, but due to presence of the novel feed additives. A tendency for greater urinary N excretion, and negative N retention for lambs fed locoweed-containing treatment versus positive N retention for CON lambs is consistent with Reed (2004), and suggests that swainsonine alters N metabolism.

Effects of Feeding Novel Product. Preliminary research (unpublished) demonstrated that novel feed additives containing a combination of bacterial cell walls, yeast, and enzymes minimized subclinical symptoms associated with swainsonine toxicity and appeared to increase the tolerance of sheep to swainsonine. Although the mode of action has not been determined, our hypothesis was that these novel feed products will increase swainsonine excretion and minimize the negative effects of locoweed on nutrient digestibility and N balance. However, the addition of AK1, AK2, or AK3 to the diets of lambs consuming locoweed did not increase the excretion of swainsonine via feces or urine. In contrast, feeding AK1 to lambs decreased swainsonine excretion in the feces and urine. Lambs fed AK2 also had lower urinary swainsonine excretion than CON lambs.

Each novel feed product contained different levels a combination of bacterial cell walls, yeast, and enzymes, with rice hulls as the carrier. Therefore, the effects of AK1, AK2, and AK3 on fiber (NDF and ADF) intake are likely due to differences in fiber concentrations between the novel feed products and the forages (locoweed and alfalfa hay). Similarly, the novel feed products contained less crude protein than locoweed and alfalfa hay, and therefore lambs fed AK1, AK2, and AK3 had lower N intake and lower grams of N digested compared with lambs fed LOCO.

CONCLUSIONS

The results demonstrate that lamb consumption of locoweed with the feed additives evaluated in the current study does not significantly affect DM and N digestibility. Lower fecal and urinary swainsonine in lambs receiving AK1 indicated that this novel feed product may affect metabolism of swainsonine in sheep.

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