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EFFICACY OF NOVEL FEED PRODUCTS TO REDUCE LOCOWEED TOXICITY IN WETHER LAMBS[1]

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ABSTRACT: Locoweed may result in impaired performance and possibly death when consumed by livestock. Novel products are needed that increase the tolerance of livestock to swainsonine, the toxicant in locoweed. The objective was to determine the efficacy of proprietary feed products to reduce locoweed toxicity in sheep. Wether lambs (n = 40; 39 ± 0.4 kg BW) were housed individually and fed 620 g/d of alfalfa hay and 100 g/d of corn-based feed in equal portions twice daily for 20 d. Lambs were equally divided into 4 BW blocks, and within block were randomly assigned to 1 of 5 treatments (randomized complete block design). Treatments were: no locoweed or feed products (CON); 20 g/d of locoweed (LOCO); 20 g/d of locoweed and 50 g/d of feed product 1 (AK1); 20 g/d of locoweed and 50 g/d of feed product 2 (AK2); 20 g/d of locoweed and 50 g/d of feed product 3 (AK3). Locoweed and feed products replaced alfalfa hay in the basal diet. Serum from venous blood was collected on d 0, 3, 6, 9, 12, 15, 18, and 20, and rumen fluid was collected on d 9 and 20 of treatment. Swainsonine was detected in serum and rumen fluid of lambs fed LOCO, AK1, AK2, and AK3, but was not detected in lambs fed CON. Serum swainsonine of lambs fed LOCO, AK1, AK2, and AK3 increased ($P < 0.05$) from d 0 to d 3, and remained elevated for the remainder of the study. Serum alkaline phosphatase was greater ($P < 0.05$) in lambs fed treatments with locoweed than CON, and was less ($P < 0.05$) in lambs fed AK3 than LOCO. Serum thyroid hormones (T3 and T4), serum total iron, and serum transferrin saturation were less ($P < 0.05$) in lambs fed treatments with locoweed than CON. Serum thyroid hormones (T3 and T4) were also lower in lambs fed AK1 than LOCO. Serum insulin was lower ($P < 0.05$) in lambs fed AK2 than LOCO. Serum urea N, and rumen fluid pH, ammonia, and total VFA were not different ($P > 0.10$) among treatments. In locoweed-fed treatments, rumen fluid swainsonine was not different ($P > 0.10$) for lambs fed AK1, AK2, or AK3 than LOCO. The results suggest that the novel feed products evaluated in the current study did not reduce serum or rumen swainsonine and had minimal effects on serum chemistry of lambs consuming locoweed.

Key words: locoweed, serum, sheep

INTRODUCTION

Locoweeds (*Astragalus* and *Oxytropis* spp.) are poisonous plants responsible for great economic losses in the livestock industry (Nielsen and James, 1992). Locoweeds contain a toxic alkaloid, swainsonine (Molyneux and James, 1982), which is produced by a fungal endophyte. Adverse effects of locoweed toxicity in animals include neurological abnormalities, emaciation, reproductive disorders, decreased performance, and death (Molyneux et al., 1985). Therefore, treatments or novel products are needed that could be supplemented to animals to decrease the toxic effects of locoweed by either decreasing gastrointestinal swainsonine availability or increasing the tolerance of livestock to swainsonine.

Previous researchers (Bachman et al., 1992; Stavanja et al., 1993; Pulsipher et al., 1994; Dugart-Stavanja et al., 1997) studied the potential for mineral supplements, zeolite clays, activated charcoal, anionic resin, and bentonite clays to bind with swainsonine in the gastrointestinal tract to alleviate locoweed toxicity in cattle, sheep, and rats. Additionally, Greenberg (1994) evaluated the possible means for ammonium chloride and diuretic compounds to increase urinary swainsonine excretion in rats and sheep. However, these studies reported minimal improvements in animals receiving treatments while exposed to locoweed. In contrast, results from preliminary research (unpublished) demonstrated that novel products containing a combination of bacterial cell walls, yeast, and enzymes decreased subclinical symptoms associated with swainsonine toxicity in sheep. Therefore, we hypothesized that supplementation of these novel products may increase livestock tolerance to swainsonine. The objective was to evaluate the effects of three proprietary feed products (Agri-King Inc., Fulton, IL) on serum concentrations of swainsonine, alkaline phosphatase, hormones, and metabolites, as well as rumen characteristics of wether lambs exposed to locoweed (Astragalus allochrous).

MATERIALS AND METHODS

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

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Animals, Design and Treatments. The experiment was conducted in the Physiology and Nutrition Building at New Mexico State University in Las Cruces, NM.

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 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 (data not presented). Lambs were individually fed a basal diet of 620 g/d of alfalfa hay and 100 g/d of corn-based feed in equal portions twice daily (0730 and 1930 h) for 20 d. Treatments (Table 1) were: no locoweed or feed products (**CON**); 20 g/d of locoweed (**LOCO**); 20 g/d of locoweed and 50 g/d of feed product 1 (**AK1**); 20 g/d of locoweed and 50 g/d of feed product 2 (**AK2**); 20 g/d of locoweed and 50 g/d of feed product 3 (**AK3**). Locoweed and feed products replaced alfalfa hay in the basal diet. 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 kilogram of live BW daily.

Sample Collections. Blood samples were collected via the jugular vein into 10 mL vacuum tubes (Corvac serum, Kendall, Ontario, CA) at 4 h after the morning feeding on d 0, 3, 6, 9, 12, 15, 18, and 20. Blood samples were stored at room temperature for 30 min to allow clotting before centrifugation (Sorvall RT600B, Thermo Electron Corp., NC) at 1,500 × g for 20 min at 5ºC. Serum was transferred 7-mL polypropylene vials and stored at -20ºC. Rumen fluid (± 100 mL) was collected via oral lavage using a suction strainer (Lodge-Ivey et al., 2009) from each animal 4 h after the morning feeding on d 9 and 20. Rumen fluid pH was measured (portable pH meter; Accumet AP72; Fisher Scientific, Pittsburg, PA) immediately, and then separated into 2 samples. For the first sample, 8 mL of rumen fluid was added to 2 mL of 25% metaphosphoric acid in polypropylene vials and stored at -20ºC for later analysis of VFA. The other rumen fluid sample was stored at -20ºC in polypropylene vials for analysis of swainsonine and ammonia.

Sample Analysis. All blood serum and rumen fluid samples were analyzed for swainsonine concentrations using the modified a-mannosidase inhibition assay as described by Taylor and Strickland (2002). Serum alkaline phosphatase was determined with a commercial kit (Amplite Calorimetric Alkaline Phosphatase Assay Kit #11950, AAT Bioquest, Inc., Sunnyvale, CA) and read on a 96-well microtiter plate reader (MRX HD, Dynex laboratories, Chantilly, VA) at 400 nm. Serum urea N concentrations were determined colorimetrically using a commercial kit (QuantiChrom Urea Assay Kit DIUR-500; Bioassay systems, Haward, CA), and serum NEFA concentrations were determined using a commercial kit (Wako NEFA HR2; Wako Chemicals USA, Inc., Richmond, VA) modified for a microplate reader at 550 nm. Serum insulin (Camacho et al., 2012), triiodothyronine (**T3**; Wells et al., 2003) and thyroxine (**T4**; Richards et al., 1999) were quantified by solid-phase RIA using commercial kits (Siemens Diagnostic, Los Angeles, CA). Serum total iron, unsaturated iron binding capacity, total iron binding capacity, and percent transferrin saturation were all analyzed using a commercial kit (Stanbio Iron and Total Iron Binding Capacity, Procedure No. 0370; Boerne, TX), and serum Fe and Cu were analyzed according to AOAC method 985.01 (AOAC, 1995). Ruminal ammonia concentrations were determined using a colorimetric assay as described by Chaney and Marbach (1962). Rumen VFA concentrations were determined in acidified rumen fluid using gas chromatography (Star 3400, Varian, Walnut Creek, CA) as described by May and Galyean (1996).

Statistical Analysis. The experiment was a randomized complete block design, and all data were analyzed as repeated measures using mixed models (SAS Inst. Inc., Cary, NC). The experimental unit was lamb. Due to a limited number (10) of 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 the random effect. An auto regressive order(1) covariance structure was specified. When treatment × day interactions were not significant ($P > 0.10$), 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. Because serum and rumen swainsonine concentrations were not detectable for CON, comparisons of locoweed treatments to CON were not evaluated statistically for swainsonine. Differences among treatments were considered significant when $P < 0.05$.

RESULTS

Lambs fed treatments containing locoweed (Astragalus allochrous) received an average of 2.4 mg swainsonine per kg of BW daily. The locoweed used in this study contained approximately 0.47% swainsonine (verified by the USDA-ARS Poisonous Plant Research Laboratory, Logan, UT).

Swainsonine was detected in serum and rumen fluid of lambs fed LOCO, AK1, AK2, and AK3, but not in lambs fed CON. Serum swainsonine of lambs fed LOCO, AK1, AK2, and AK3 increased ($P < 0.05$) from d 0 to d 3, and remained elevated for the remainder of the study (data not shown). No treatment × day interactions ($P > 0.10$) were detected for all response variables that were measured in serum and rumen fluid. Therefore, all serum and rumen fluid data in Table 2 are least squares means (± SE) for treatment main effects. Lambs fed treatments containing locoweed (LOCO, AK1, AK2, and AK3) had greater ($P < 0.05$) serum alkaline phosphatase and unsaturated iron binding capacity, and had lower ($P < 0.05$) serum T3 and

Table 1. Dietary treatments fed to lambs

Item	Treatments				
	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	148.8	145.1	150.3	122.9

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

Table 2. Serum concentrations of swainsonine, alkaline phosphatase (ALP), hormones, and metabolites, and rumen concentrations of swainsonine, ammonia (NH_3), total VFA and pH of lambs exposed to locoweed toxicity and supplemented with novel feed products

	Treatments[1]						Contrasts[2]			
	CON	LOCO	AK1	AK2	AK3	SEM	CON vs other	LOCO vs AK1	LOCO vs AK2	LOCO vs AK3
Serum[3]										
Swainsonine, µg/mL	-	0.47	0.41	0.46	0.43	0.04	-	0.06	0.79	0.17
ALP, mU/mL	106	529	455	652	257	57.8	<0.01	0.34	0.11	<0.01
Urea N, mg/dL	45.4	47.0	50.6	46.0	48.1	3.29	0.14	0.09	0.65	0.59
NEFA, mEq/L	0.15	0.24	0.20	0.17	0.21	0.04	0.06	0.16	0.04	0.31
Insulin, ng/mL	0.24	0.24	0.23	0.17	0.21	0.03	0.14	0.67	<0.01	0.21
T3, ng/mL	0.93	0.69	0.58	0.62	0.63	0.05	<0.01	0.01	0.10	0.14
T4, ng/mL	63.5	43.3	38.5	41.9	38.1	2.49	<0.01	0.01	0.49	<0.01
Total Iron, µg/dL	166	138	145	135	143	22.9	<0.01	0.54	0.74	0.69
UIBC, µg/dL	110	127	148	146	139	14.2	<0.01	0.01	0.03	0.17
TIBC, µg/dL	271	264	293	282	283	36.2	0.40	0.05	0.24	0.21
TF saturation, %	59.1	50.8	48.2	47.0	49.5	2.12	<0.01	0.18	0.05	0.49
Fe, ppm	1.46	1.12	1.13	1.10	1.06	0.18	<0.01	0.94	0.87	0.59
Cu, ppm	0.61	0.62	0.63	0.61	0.61	0.08	0.83	0.82	0.88	0.80
Rumen[4]										
Swainsonine, µg/mL	-	7.19	6.97	7.39	7.65	0.49	-	0.71	0.74	0.47
pH	6.72	6.79	6.79	6.73	6.78	0.06	0.39	0.97	0.47	0.91
NH_3, mM	11.1	10.0	11.3	10.0	11.5	1.04	0.68	0.29	0.97	0.20
Total VFA, mM	111	113	110	109	112	5.31	0.93	0.67	0.56	0.87

[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.
[2]Fixed effects of treatment × day interaction were not significant ($P > 0.10$) for all response variables, and 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.
[3]Blood samples were collected from the jugular vein of each animal at 4 h after the morning feeding on d 0, 3, 6, 9, 12, 15, 18, and 20.
T3 = triiodothyronine; T4 = thyroxine; UIBC = unsaturated iron binding capacity; TIBC = total iron binding capacity; TF saturation = transferrin saturation.
[4]Rumen fluid samples were collected from each animal at 4 h after morning feeding on d 9 and 20 via oral lavage with a strainer attached to a manual suction pump (Lodge-Ivey et al., 2009).

T4, serum total iron, serum transferrin saturation, and serum Fe compared with lambs fed CON (Table 2). Lambs fed AK1 had decreased ($P < 0.05$) serum T3 and T4, and greater ($P < 0.05$) unsaturated iron binding capacity and total iron binding capacity than lambs fed LOCO. Lambs fed AK2 had lower ($P < 0.05$) serum NEFA, serum insulin, and transferrin saturation, and had greater ($P < 0.05$) unsaturated iron binding capacity than lambs fed LOCO. Lambs fed AK3 had lower ($P < 0.05$) serum alkaline phosphatase and T4 than lambs fed LOCO. Serum urea N, and rumen fluid pH, ammonia, and total VFA were not different ($P > 0.10$) among treatments. In locoweed-fed treatments, rumen fluid swainsonine was not different ($P > 0.10$) for lambs fed AK1, AK2, or AK3 than LOCO.

DISCUSSION

Effects of Feeding Locoweed. Lambs fed diets containing locoweed had peak serum concentrations of swainsonine by the first blood collection (d 3) after locoweed exposure. Taylor and Strickland (2002) reported that swainsonine can be detected in blood within hours after animals have consumed locoweed. In the current study, serum swainsonine concentrations were similar to that reported by Stegelmeyer et al. (1995) for sheep fed alfalfa with locoweed at 1.5 mg swainsonine per kg BW.

Serum alkaline phosphatase of lambs fed locoweed was approximately 5-fold greater than CON lambs, which is similar to that reported by Ortiz et al. (1997). Elevated serum alkaline phosphatase indicated acute swainsonine intoxication of lambs, which is likely due to defective glycoprotein processing (Reed, 2004). Lower concentrations of T3 and T4 in lambs fed locoweed than CON are consistent with previous research (Pulsipher et al., 1994; Ortiz et al., 1997; Obeidat et al., 2005b). The effects of swainsonine on cytoplasmic vaculation and tissue death (Molyneux and James, 1982) may cause a thyroid gland abnormality leading to decreased T3 and T4 production.

Decreased serum total iron in locoweed-fed lambs indicates the possible effect of swainsonine on Fe metabolism, mobilization, and transport from its storage in the liver (Reed, 2004) or an indication of renal damage or anemia (Bachman et al., 1992). Serum transferrin saturation, which is the percent of Fe saturation on the α1- glycoprotein transferrin (Reed, 2004) was less in lambs fed treatments with locoweed than CON, perhaps due to altered glycoproteins from swainsonine toxicity. Serum unsaturated iron binding capacity was greater in lambs fed locoweed-containing diets than CON, which indicates less Fe was being bound to transferring sites. Blood urea N was not affected by feeding locoweed to lambs, which is consistent with previous research (Pulsipher et al., 1994; Taylor et al., 2000). A tendency for greater serum NEFA concentrations in lambs fed locoweed compared with CON is an indication of altered energy metabolism, and is consistent with Obeidat et al. (2004). Rumen fluid pH, ammonia, and total VFA concentrations were not different among treatments, and demonstrate that swainsonine has minimal impact on anaerobic microbial fermentation in the rumen. These results are consistent with the results of Reed (2004) and Obeidat et al. (2005a).

Effects of Feeding Novel Products. Although the mechanism of action is not clear, results from preliminary research (unpublished) demonstrated that novel products containing a combination of bacterial cell walls, yeast, and enzymes decreased some of the liver enzymes associated with swainsonine toxicity in sheep. Therefore, our hypothesis was that supplementation of these novel products will increase the tolerance of livestock to swainsonine.

The tendency for serum swainsonine concentrations to be lower for lambs fed AK1 than LOCO could be an indication of decreased swainsonine absorption from the gastrointestinal tract or greater swainsonine removal from the blood. However, excretion of swainsonine in feces and urine was not greater for lambs fed AK1 than LOCO. Also, reduced thyroid hormones (T3 and T4) and greater unsaturated iron binding capacity and total iron binding capacity in lambs fed AK1 than LOCO suggest greater level of toxicity, which indicates that AK1 did not increase the tolerance of lambs to swainsonine.

Serum concentrations of swainsonine and alkaline phosphatase were not different between lambs fed AK2 and LOCO, which is an indication that AK2 did not increase the tolerance of lambs to swainsonine. Lower concentrations of NEFA and insulin indicate improved energy metabolism in lambs fed AK2 than LOCO, but greater unsaturated iron binding capacity and lower transferrin saturation indicates decreased Fe transportation which may lead to anemia.

Serum concentrations of swainsonine were not different, but lower serum alkaline phosphatase in lambs fed AK3 versus LOCO indicate less damage from swainsonine consumption. However, lower serum T4 in lambs fed AK3 compared with LOCO does not support increased tolerance to swainsonine.

CONCLUSIONS

Greater concentration of serum swainsonine and alkaline phosphatase, and lower serum thyroid hormones, serum total iron, and serum transferrin saturation in lambs fed treatments with locoweed compared with control lambs are indicative of swainsonine toxicity. No differences in rumen fermentation characteristics indicated that locoweed consumption had little impact on anaerobic microbial fermentation. Limited positive changes in serum swainsonine, alkaline phosphatase, hormones, and metabolites indicated that the novel feed products evaluated in the current study had minimal effects on improving serum chemistry of wether lambs with locoweed poisoning.

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