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VISCERAL ORGAN MASS AND JEJUNUM CELL PROLIFERATION OF LAMBS FED ALFALFA HAY, LOCOWEED, AND FEED ADDITIVES[1]

F. A. Allataifeh, M. N. Sawalhah, S. A. Soto-Navarro, and C. A. Löest

New Mexico State University, Las Cruces, NM

ABSTRACT: Swainsonine toxicity causes organ damage, decreases production, and may alter digestive function of livestock consuming locoweed. Therefore, novel products are needed to improve animal tolerance to swainsonine. This study evaluated effects of 3 novel feed products on sheep visceral organ mass and jejunum cell proliferation. Forty wether lambs (39 ± 0.4 kg BW) were divided into 4 BW blocks, and randomly assigned within block to 5 treatments. Lambs were individually fed 620 g/d of alfalfa hay and 100 g/d of corn-based feed twice daily in equal portions for 20 d. 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 (contained bacterial cell walls, yeasts, and enzymes) replaced alfalfa hay in the diet. After a 24 h fast, 4 randomly selected lambs from each treatment were euthanized on d 21. Data were analyzed using mixed models and contrasts to compare CON with the average for treatments containing locoweed (LOCO, AK1, AK2, and AK3), LOCO vs. AK1, LOCO vs. AK2, and LOCO vs. AK3. Hot carcass weights were lower ($P < 0.05$) for lambs fed treatments with locoweed compared with CON. Fasting BW and HCW were lower ($P < 0.05$) for lambs fed AK2 than LOCO. Treatment did not affect ($P \geq 0.09$) carcass dressing percentage and weights (relative to fasting BW) of jejunum, total small intestine, pancreas, spleen, and heart. Weights (relative to fasting BW) of the rumen complex, duodenum, large intestine, liver, kidney, and lung were greater ($P < 0.05$), and ileum weights were smaller ($P < 0.05$) in lambs fed treatments with locoweed than CON. However, these organ weights were not different ($P \geq 0.18$) for lambs fed AK1, AK2, or AK3 compared with LOCO. Mesenteric fat was less ($P < 0.05$) in lambs fed AK2 than LOCO, and jejunum cell proliferation was greater ($P < 0.05$) in lambs fed AK1 than LOCO. Results suggest that the novel feed products evaluated in the current study did not minimize the visceral organ weight changes associated with swainsonine toxicity in sheep.

Key words: organ weight, sheep, swainsonine

INTRODUCTION

Locoweeds (*Astragalus* and *Oxytropis* spp.) are poisonous plants that contain the toxic alkaloid, swainsonine (Molyneux and James, 1982). Swainsonine inhibits intracellular α-mannosidases, causes oligosaccharides to accumulate in lysosomes, and alters the processing of glycoproteins in animal cells (Dorling et al., 1980; Kang et al., 1993). Accumulation of glycoproteins and oligosaccharides causes intracellular vacuolization in the brain, liver, kidneys, and other tissue (James et al., 1969; Orgad et al., 1985; Novikoff et al., 1985). Animals exposed to swainsonine have enlarged organs, such as the liver, heart, kidneys, spleen, and testes, when compared to their control counterparts (Dugart-Stavanja et al., 1997). The adverse effects of locoweed toxicity in livestock include neurological abnormalities, emaciation, reproductive disorders, decreased performance, and death (Molyneux et al., 1985).

Because of the adverse effect of locoweed toxicity, livestock producers could benefit from products, feed additives, or supplements that potentially increase the tolerance of animals to swainsonine. However, previous studies (Bachman et al., 1992; Stavanja et al., 1993a; Greenberg, 1994; Pulsipher et al., 1994; Richards et al., 1999; Dugart-Stavanja et al., 1997) reported little or no beneficial effects of supplementing minerals, zeolite clays, activated charcoal, anionic resin, and bentonite clays to cattle, sheep, and rats exposed to swainsonine. In contrast, preliminary data from an unpublished study indicated that feeding novel feed products containing combinations of bacterial cell walls, yeast, and enzymes could minimize the negative effects of swainsonine on rumen epithelial cells and organ weights of sheep fed locoweed. Therefore, the objective of this experiment was to evaluate the effects of three novel feed products (Agri-King Inc., Fulton, IL) on visceral organ mass and jejunum cell proliferation of wether lambs exposed to locoweed (*Astragalus allochrous*).

MATERIALS AND METHODS

Animals, Design, and Treatments

The Institutional Animal Care and Use Committee of New Mexico State University approved all experimental procedures. In a randomized complete block design, 40 wether lambs (39 ± 0.4 kg initial BW) were equally divided into 4 blocks based on their BW. Within each block, lambs were randomly assigned to 1 of 5 dietary treatments. Treatments (Table 1) were a control diet (86% alfalfa hay

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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 product 1 replacing alfalfa hay (**AK1**), LOCO with 50 g/d of feed product 2 replacing alfalfa hay (**AK2**), and LOCO with 50 g/d of feed product 3 replacing alfalfa hay (**AK3**). The novel feed products (Agri-King Inc., Fulton, IL) consisted of combinations of bacterial cell walls, yeast, and enzymes, with rice hulls as the carrier. Locoweed (*Astragalus allochrous*) was collected in April in New Mexico and chopped (The Western Bear Cat No 5A, by Western Land Roller Co., Hastings, NE, USA) to reduce particle size. The amount of locoweed fed was calculated to supply approximately 2 mg swainsonine per kg of BW daily. Lambs were housed in individual feeding pens from d 1 to 14 for adaptation to the dietary treatments, and in metabolism crates from d 15 to 20 for urine and fecal collections to determine nutrient digestibility and N retention (data presented previously by Allataifeh et al., 2012).

Sample Collections

After lambs were fed the dietary treatments for 20 d, 5 of 10 lambs in each block were randomly selected (one lamb per treatment in each block) and removed from feed and water for 24 h before euthanasia. Fasting BW was recorded, and lambs were then euthanized using a captive bolt stunner followed by exsanguination (according to standard procedures at the New Mexico State University Meat Laboratory). Immediately after evisceration, HCW and weights of visceral organs (stomach, small intestines, large intestines, heart, lungs, liver, pancreas, spleen, kidneys) and mesenteric fat were recorded. The duodenum was identified from the pylorus to where the gastrosplenic vein entered the mesenteric vein, the jejunum was identified as the segment from the end of the duodenum to the jejunum and ileum junction, and the ileum was identified as the remaining small intestinal segment to the ileocecal junction (Soto-Navarro et al., 2004). The jejunum was sampled in 10-cm cross section from the midpoint as described previously (Swanson et al., 1999; Soto-Navarro et al., 2004) and prepared for cellular proliferation analysis (Reynolds and Redmer, 1992).

Statistical Analysis

The experiment was a randomized complete block design (4 complete blocks based on BW and date of euthanasia), and all data were analyzed using mixed models (SAS Inst. Inc., Cary, NC) with lamb as the experimental unit. The statistical model included treatment as fixed effect and block was random. 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

Fasting BW tended to be lower ($P = 0.07$), and HCW were lower ($P < 0.05$) for lambs fed treatments containing locoweed compared with CON lambs (Table 2). Fasting BW and HCW were lower ($P < 0.05$) for lambs fed AK2 than LOCO. Treatment did not affect ($P \geq 0.09$) carcass dressing percentage and weights (relative to fasting BW) of jejunum, total small intestine, pancreas, spleen, and heart. Weights (relative to fasting BW) of the rumen complex, duodenum, large intestine, liver, kidney, and lung were greater ($P < 0.05$), and ileum weights were smaller ($P < 0.05$) in lambs fed treatments with locoweed than CON. However, these organ weights were not different ($P \geq 0.18$) for lambs fed AK1, AK2, or AK3 compared with LOCO. Mesenteric fat was less ($P < 0.05$) in lambs fed AK2 than LOCO, and jejunum cell proliferation was greater ($P < 0.05$) in lambs fed AK1 than LOCO.

DISCUSSION

Effects of Feeding Locoweed

Lambs with a daily exposure of approximately 2 mg swainsonine per kg of BW had greater rumen complex, duodenum, liver, kidney, and lung weights (relative to fasting BW) than CON lambs, which is consistent with increases in the relative size of visceral organs when rats were exposed to 3.7 or 7.7 mg swainsonine per kg of BW (Dugart-Stavanja et al., 1997). In the current study, feed intake was restricted at 1.8% of BW for all treatments (nutrient intakes were presented previously by Allataifeh et al., 2012). Therefore, increases in relative visceral organ mass were likely due to swainsonine toxicosis and not because of the potential effect of nutrient intake on visceral organ size. According to Stavanja et al. (1992; 1993b), increases in the relative organ size is a good toxicosis index of animals exposed to swainsonine, particularly when compared to control animals with similar feed intake. However, Stegelmeier et al. (1999) reported no effects on the organ weights of sheep exposed to 0.80 mg or less of swainsonine per kg of BW. Therefore, increases in visceral organ size appear to occur only at high exposure to swainsonine, and may be due to tissue vacuolization, protein accumulation, cell lysis, and inflammation.

Effects of Feeding Novel Product

Preliminary research (unpublished) indicated that supplementation of novel feed products containing combinations of bacterial cell walls, yeast, and enzymes minimized the negative effects of swainsonine on rumen and liver weight of sheep fed locoweed. However, these responses were not observed in the current study. Less mesenteric fat in lambs fed AK2 than LOCO could be due to dietary energy dilution, because the carrier ingredient of the novel feed product was high fiber rice hulls (Allataifeh et al., 2012).

Conclusions

Greater relative weights for the rumen complex, duodenum, liver, kidneys, and lungs of lambs fed treatments with locoweed compared with control lambs are indicative of swainsonine toxicity. The novel feed products evaluated in the current study did not minimize the visceral organ weight changes associated with swainsonine toxicity in sheep.

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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 α-mannosidase inhibition assay as described by Taylor and Strickland (2002).

Table 2. Fasting BW, HCW, visceral organ mass, and jejunum cell proliferation of lambs after 20 d of exposure to locoweed toxicity and supplemented with novel feed additives

	Treatments[1]						Contrasts[2]			
	CON	LOCO	AK1	AK2	AK3	SEM	CON vs other	LOCO vs AK1	LOCO vs AK2	LOCO vs AK3
No of lambs	4	4	4	4	4					
Fasting BW, kg	33.4	32.9	31.6	30.5	31.9	0.76	0.07	0.26	0.04	0.39
HCW, kg	17.3	16.8	15.8	15.1	16.0	0.43	0.01	0.12	0.02	0.19
Dressing %	51.7	51.3	50.0	49.5	50.0	0.75	0.09	0.25	0.11	0.23
Visceral organs, g/kg fasting BW										
Rumen complex[3]	24.0	27.0	28.9	27.7	28.3	1.53	0.04	0.40	0.76	0.56
Duodenum	1.02	2.00	2.18	1.73	2.00	0.32	0.02	0.66	0.61	0.93
Jejunum	13.8	13.5	13.4	14.0	11.2	1.23	0.57	0.94	0.79	0.22
Ileum	0.71	0.50	0.45	0.49	0.43	0.08	0.02	0.69	0.92	0.59
Small intestine	15.5	16.0	16.0	16.2	13.7	1.24	0.95	0.98	0.90	0.22
Large intestine	12.5	14.0	14.3	15.0	13.9	0.53	<0.01	0.71	0.22	0.91
Lungs	10.9	12.9	13.1	12.7	12.0	0.58	0.01	0.86	0.82	0.30
Liver	12.9	14.2	15.2	14.6	14.8	0.48	<0.01	0.18	0.57	0.39
Kidneys	2.76	3.15	3.38	3.22	3.10	0.12	<0.01	0.21	0.70	0.75
Heart	4.97	4.52	5.06	4.98	4.98	0.27	0.78	0.18	0.24	0.25
Spleen	1.78	1.78	1.88	1.83	1.61	0.08	0.96	0.38	0.69	0.17
Pancreas	1.19	1.37	1.39	1.50	1.59	0.15	0.12	0.90	0.55	0.31
Messenteric fat	19.7	20.1	17.3	15.8	17.0	1.04	0.09	0.09	0.01	0.06
Jejunum cell prolif., %	3.70	4.07	7.67	2.92	4.23	1.31	0.43	0.04	0.51	0.93

[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]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]Rumen complex includes the rumen, reticulum, omasum, and abomasum.