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Plasma amino acids of wether lambs supplemented with novel feed products to reduce locoweed toxicity[1]

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ABSTRACT: Locoweeds impair performance and may cause death in grazing livestock. Novel feed products are needed that counter or minimize the toxic effects of locoweed. The objective was to evaluate effects of 3 feed product formulations on plasma AA of lambs consuming locoweed. Forty wether lambs (39 ± 0.4 kg BW) were housed individually and fed 620 g/d of alfalfa hay and 100 g/d of corn-based feed twice daily in equal portions for 20 d. Lambs were equally divided into 4 blocks, and randomly assigned to 1 of 5 treatments within each block. 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. Plasma from jugular venous blood was collected on d 0, 3, 6, 9, 12, 15, 18, and 20 of treatment. Treatment × day interactions ($P < 0.05$) were detected for plasma Gly and Thr, but not for other AA ($P > 0.05$). Plasma Gly was not different among treatments on d 0, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 6, 9, 12, 15, 18, and 20. Plasma Thr was not different among treatments on d 0 and 6, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 9 (except AK2), 12 (only AK2), 15, 18, and 20. Plasma, Leu, Met, Val, Ala, Asn, and Pro were greater ($P < 0.05$), while Glu was lower ($P < 0.05$) in lambs fed treatments containing locoweed compared with CON. The increase in plasma AA in lambs fed locoweed suggests that AA uptake was impaired and(or) tissue protein degradation was increased. Plasma concentrations of His, Ile, Lys, Phe, Trp, Asp, Gln, Ser, and Tyr were not different ($P \geq 0.07$) between lambs fed treatments containing locoweed and CON. Lambs supplemented with AK1, AK2, or AK3 had plasma AA that were not different ($P \geq 0.07$) than lambs fed LOCO. We conclude that locoweed consumption alters plasma AA in lambs and that addition of novel formulations did not counter the effects of locoweed on plasma AA.

Key words: amino acids, locoweed, sheep, swainsonine

INTRODUCTION

Locoweeds are poisonous legumes that are responsible for substantial economic losses in livestock (Nielsen and James, 1992). Swainsonine, a fungal endophyte alkaloid (Cook et al., 2011), is the primary toxicant in locoweed (Molyneux and James, 1982). Swainsonine alters glycoprotein processing, which results in a number of subclinical and clinical disorders in livestock consuming locoweed. Many proteins that are essential for AA metabolism are glycosylated, such as rBAT and 4F2 (Mailliard et al., 1995). Swainsonine-induced alteration of essential glycoproteins could impair AA metabolism in livestock consuming locoweed (Pan et al., 1993).

Novel feed products are needed that counter or minimize the toxic effects of locoweed. For example, a bacterial strain (YLZZ-1) isolated from the soil surrounding *Oxytropis kansuensis* was found to degrade swainsonine in culture (Zhao et al., 2009). Furthermore, a novel feed supplement containing a combination of bacterial cell walls, yeast, and enzymes decreased subclinical symptoms of locoweed toxicity in sheep (personal communication, A. Temple, 2010).

We hypothesized that consumption of locoweed will alter AA metabolism in lambs and such alterations can be countered by feeding novel products. The objective was to evaluate the effects of 3 Agri-King Inc. (Fulton, IL) feed product formulations on plasma AA concentrations of wether lambs consuming locoweed.

MATERIALS AND METHODS

Experimental Design, Animals, and Treatments

Experimental procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. The experimental design was a randomized complete block, with 4 experimental periods (block) and 5 treatments. Forty wether lambs (initial BW = 39.0 ± 0.4 kg) were randomly and equally assigned to 4 experimental periods. Within each experimental period, lambs were randomly assigned to 1 of 5 dietary treatments. Treatments (Table 1) were: no locoweed or feed products (**CON**); 20

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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 (Agri-King Inc., Fulton, IL) 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 α-mannosidase inhibition assay as described by Taylor and Strickland (2002).

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**). Treatments were mixed with a basal diet (620 g/d of alfalfa hay and 100 g/d of corn-based feed); locoweed and feed products replaced alfalfa hay in the basal diet. Mixed treatment diets were fed individually in equal portions twice daily (0730 and 1930) for 20 d. Lambs 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 (Allataifeh et al., 2012a).

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, Western Land Roller Co., Hastings, NE) to reduce particle size. The locoweed contained approximately 0.47% swainsonine (also verified by the USDA-ARS Poisonous Plant Research Laboratory, Logan, UT), which resulted in a daily swainsonine dose of 2 mg·kg BW-1 for lambs fed locoweed.

Sample Collections and Analysis

Jugular vein blood samples were collected (10-mL Monoject sodium heparin tubes) at 4 h after the morning feeding on d 0, 3, 6, 9, 12, 15, 18, and 20. Samples were centrifuged (1,500 × g for 20 min at 5ºC; Sorvall RT6000B, Thermo Electron Corp., NC), and plasma was transferred into 7-mL polypropylene vials and stored at -20ºC for AA analysis.

Plasma AA concentrations were determined using a commercially available kit (EZ:faast ref. No. KG0-7165, Phenomenex, Torrance, CA) via GLC (CP-3800, Varian, Walnut Creek, CA). This kit supplied the GC column, reagents, preparation vials and racks, and standards. The mixture of eluted sample was allowed to separate into 2 layers. The upper organic layer was used for AA analysis by GC, with 2 μL injection volume, 250ºC inlet temperature, carried by a constant flow (1.5 mL/min) of helium gas, with 320ºC for the detector. This procedure is described by Waggoner et al. (2009).

Statistical Analysis

The experiment was a randomized complete block design, and data were analyzed as repeated measures using mixed models (SAS Inst. Inc., Cary, NC). The experimental unit was lamb and experimental period served as the blocking factor. The statistical model included treatment, day, and treatment × day interaction as fixed effects, and experimental period was the random effect. Compound symmetry covariance structure was specified for repeated measures. 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. Differences among treatments were considered significant when $P < 0.05$.

RESULTS

Treatment × day interactions ($P < 0.05$) were detected for plasma Gly and Thr concentrations (Fig. 1). Plasma Gly concentration was not different among treatments on d 0, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 6, 9, 12, 15, 18, and 20. Plasma Thr was not different among treatments on d 0 and 6, but was greater for LOCO, AK1, AK2 and AK3 than CON on d 3 (except AK1 and AK2), 9 (except AK2), 12 (only AK2), 15, 18, and 20. Treatment × day interactions were not detected for other plasma AA ($P > 0.05$).

Plasma essential AA, Leu, Met, Val, and nonessential AA, Ala, Asn, and Pro (Table 2), were greater ($P < 0.05$), while the concentration of Glu was lower ($P < 0.05$) in lambs fed locoweed compared with CON. Plasma His, Ile, Lys, Phe, Trp, Asp, Gln, Ser, and Tyr were not different ($P \geq 0.07$) between lambs fed treatments containing locoweed and CON. Plasma AA in lambs supplemented with AK1, AK2, or AK3 were not different ($P \geq 0.07$) than plasma AA in LOCO lambs.

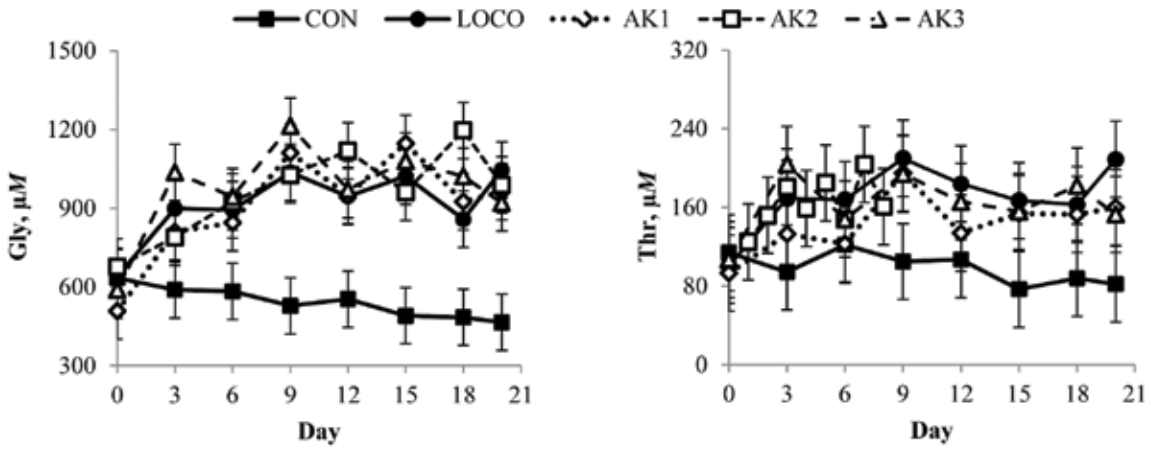

Figure 1. Plasma Gly and Thr of lambs exposed to locoweed toxicity and supplemented with novel feed products. Treatment × day interaction ($P < 0.05$) was detected for Gly and Thr of lambs exposed to locoweed toxicity and supplemented with novel feed products. Treatments were no locoweed or feed products (CON); 20 g/d of locoweed (LOCO); 20 g/d of locoweed and 50 g/d of feed product 1 (AK1; Agri-King Inc. Fulton, IL); 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). Treatments were mixed with a basal diet (620 g/d alfalfa hay and 100 g/d corn-based feed); locoweed and feed products replaced alfalfa hay in the basal diet.

Table 2. Plasma AA of lambs exposed to locoweed toxicity and supplemented with novel feed products[1]

	Treatments[2]						Contrasts			
Item	CON	LOCO	AK1	AK2	AK3	SEM	CON vs Other	LOCO vs AK1	LOCO vs AK2	LOCO vs AK3
Essential AA[3], μ*M*										
His	45.1	38.3	39.0	42.6	45.1	2.95	0.25	0.87	0.30	0.11
Ile	59.7	68.1	61.1	64.6	67.2	5.14	0.13	0.13	0.44	0.83
Leu	86.2	109	94.5	101	104	9.09	0.01	0.07	0.31	0.54
Lys	38.8	34.3	33.8	34.3	36.2	4.15	0.07	0.86	0.98	0.51
Met	11.9	17.2	15.2	15.4	15.3	1.55	<0.01	0.09	0.12	0.10
Phe	35.0	38.6	35.6	37.8	41.2	2.92	0.20	0.35	0.78	0.42
Trp	29.4	26.2	23.7	26.8	28.3	4.17	0.16	0.38	0.84	0.46
Val	147	181	157	170	172	17.0	0.02	0.05	0.34	0.45
Nonessential AA[4], μ*M*										
Ala	189	231	207	224	219	20.4	0.04	0.19	0.69	0.54
Asp	2.27	1.62	1.57	1.89	2.06	0.43	0.20	0.91	0.55	0.35
Asn	33.6	42.9	38.6	42.7	46.3	6.00	<0.01	0.27	0.96	0.38
Glu	198	161	148	159	162	16.9	<0.01	0.45	0.90	0.95
Gln	205	223	206	243	248	20.9	0.19	0.49	0.38	0.29
Pro	101	137	125	132	133	12.9	<0.01	0.15	0.51	0.62
Ser	163	209	177	206	216	29.7	0.07	0.22	0.90	0.80
Tyr	44.6	49.6	42.5	47.8	49.0	3.57	0.43	0.10	0.68	0.90

[1]Blood samples were collected from the jugular vein of each animal at 4 h after feeding on d 0, 3, 6, 9, 12, 15, 18, and 20.
[2]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; Agri-King Inc. Fulton, IL); 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). Treatments were mixed with a basal diet (620 g/d alfalfa hay and 100 g/d corn-based feed); locoweed and feed products replaced alfalfa hay in the basal diet.
[3]Does not include plasma concentrations of Arg. A significant treatment × day interaction ($P < 0.05$) for Thr.
[4]Does not include plasma concentrations of Cys. A significant treatment × day interaction ($P < 0.05$) for Gly.

DISCUSSION

Effects of Feeding Locoweed

Plasma essential and non-essential AA were found in greater concentrations in lambs fed locoweed compared with CON lambs. Allataifeh et al. (2012a) reported a negative N balance for lambs fed locoweed compared with lambs not fed locoweed. This suggested that swainsonine, from locoweed consumption, may have increased catabolic pathways in AA metabolism. In the current study, the greater plasma AA concentrations observed for all groups fed locoweed versus CON was perhaps because of a decrease in the uptake of plasma AA for protein synthesis. This could result in greater deamination of AA, which is supported by the decreased N retention reported in the study of Allataifeh et al. (2012a).

The negative N balance for locoweed-fed lambs reported by Allataifeh et al. (2012a) could also imply that there was an increase in tissue protein degradation, which would result in an increase in AA into the blood. A tendency for serum urea N to increase and a significant increase in NEFA (Allataifeh et al., 2012b) is a very convincing argument that mobilization of both tissue protein and fat stores occurred in lambs fed locoweed versus CON animals (Allataifeh et al., 2012b).

Effects of Feeding Novel Products

Unpublished results (personal communication, A. Temple, 2010) indicated that novel product formulations containing a combination of bacterial cell walls, yeast, and enzymes seemed to alleviate locoweed toxicity in sheep. However, our data indicated that addition of novel formulations containing various combinations of the same products did not counter effects of locoweed consumption on plasma AA.

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