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Glucomannan as a Dietary Ergot Alkaloid Adsorbent for Mares

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Abstract: Glucomannan modified for greater ergot alkaloid affinity was fed to mares to evaluate its efficacy as a dietary ergot alkaloid adsorbent. Mares were fed bermudagrass hay along with one of four feed mixtures containing either endophyte-free (E-) or toxic endophyte-infected tall fescue seed. All rations based on toxic endophyte-infected tall fescue seed contained 1.69 ppm ergot alkaloids on a dry matter (DM) basis. Modified GLMN was provided at 0 (E+), 5 (E+5), or 10 g (E+10) twice daily. Efficacy of ergot alkaloid adsorption by GLMN was evaluated by measuring alkaloid consumption vs. fecal and urine excretion. Blood metabolites and hormones were evaluated as physiological indicators of ergot elimination. Hay and total DM intake (DMI) was not affected ($p = 0.59$) and ranged from 1.17 to 1.33 and 2.0 to 2.15% BW/d, respectively. Ergot alkaloid concentrations in feces ($p = 0.02$; 31.02 to 225.53 ng g^{-1}) and urine ($p < 0.01$; 0.96 to 37.12 ng mg^{-1} creatinine) were greater for mares receiving E+, E+5, or E+10 than E- and supplemental GLMN did not alter urinary ergot alkaloid excretion. No differences were found within treatment phase for plasma 3,4-dihydroxyphenylacetic acid (DOPAC; $p = 0.24$; 4.94 to 8.68 ng mL^{-1}), serum cortisol ($p = 0.14$; 4.48 to 5.69 μg dL^{-1}), or PRL ($p = 0.40$; 2.89 to 3.85 ng mL^{-1}). Findings are inconclusive and further investigation is needed to determine the efficacy of feeding modified GLMN to mares grazing toxic endophyte-infected tall fescue pastures.

Key words: Endophyte, ergot alkaloid, glucomannan, mare, tall fescue

INTRODUCTION

Tall fescue (*Lolium arundinacea* (L.) Schreb.) is an economically important cool season forage in the United States of America, but most tall fescue pastures are infected with a fungal endophyte, *Neotyphodium coenophialum*[1]. While toxic ergot alkaloid-producing endophyte has enabled tall fescue to persist under drought and increased grazing pressure, its detrimental effects upon growth and reproduction in livestock and horses has been well documented[2-4]. Novel endophyte-infected varieties offer similar benefits to the plant without leading to adverse health conditions of livestock, but persistence of novel varieties may be less than toxic endophyte-infected tall fescue[5]. Further, initial investments required to replace a stand of toxic endophyte-infected tall fescue may not be economical for many farmers. Until novel varieties of fescue are better adapted to a wider region and varying production conditions, methods to manage forage and livestock for reducing consequences of excessive ergot alkaloid consumption should be emphasized. Unlike ruminants, where dilution of ergot alkaloids has been achieved by over-seeding pastures with clovers, perennial grasses, or by *ad libitum* access to non-endophytic hay, thereby diminishing adverse affects caused from ergot alkaloid consumption. Similar attempts to dilute ergot alkaloids within the diet of grazing horses has not been as effective[6,7]. Further, ergot alkaloid exposure by ruminants has been reduced by feeding a modified yeast-derived glucomannan (GLMN), which adsorbs ergot alkaloids reducing their bioavailability and thereby increasing fecal excretion[8]. Previously, a GLMN variant was used to adsorb *Fusarium* mycotoxins in pigs, chickens and

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horses[9-11], but no attempt has been made to assess ergot alkaloid adsorption by GLMN in diets of equine. Objective of the current project was to evaluate ergot alkaloid-specific GLMN affect on urinary and fecal ergot alkaloid excretion by non-gravid, non-lactating mares consuming toxic endophyte-infected tall fescue seed.

MATERIALS AND METHODS

Experimental design: A randomized complete block design consisting of three phases: initial (1 d), treatment (12 d) and post-treatment (14 d) was conducted at Leveck Animal Research Center in Starkville, Mississippi located in a temperate region of southern USA. The 27-d trial comprised of 20 open mares (498±31.5 kg) randomly assigned to treatments and blocked by barn to provide five replications per treatment. During initial and treatment phases, mares were housed in individual stalls at two adjacent barns. During post-treatment phase, mares continuously grazed bermudagrass (*Cynodon dactylon* (L.) Pers.) pasture.

During treatment phase, mares consumed bermudagrass hay along with a mixed feed (offered at 0.8% of initial BW, DM basis) consisting of 40% commercially available sweet feed (Horse and Cattle 10% Sweet Feed; Cargill Animal Nutrition, Minneapolis, MN), 50% whole, unprocessed tall fescue seed and 10% molasses (as fed basis). Tall fescue seed (Pennington Inc., Madison, GA) was either toxic endophyte-infected (3.50 ppm ergot alkaloid/seed, DM basis; Table 1) or endophyte free (E-). Mixed feed containing toxic endophyte-infected seed included 1.69 ppm ergot alkaloids (DM basis) and 0 (E+), 5 (E+5), or 10 g (E+10) GLMN, top-dressed twice daily. Hay was offered free choice and mixed feed was provided at 0.8% BW (DM basis) twice daily. Each morning at 0700, hay and feed intake was measured and orts were discarded. After collecting blood, fecal and/or urine samples, mares were segregated by treatment (E+ or E-) and released into two conjoining dry lots (0.404 ha each) for approximately 6 h/d, having access to only water. After 12-d collection period, mares were removed from treatments and placed on bermudagrass pasture for 14 d to evaluate post treatment responses.

Sample collection: Mixed feed, hay and orts were weighed and sampled for nutrient analysis, with exception of refused hay, which was not sampled. Every other day, two blood samples from each mare were taken by jugular venipuncture. One vial contained EDTA and 240 μL reduced glutathione (60 mg L^{-1}) to serve as an anticoagulant[12]. Blood samples were immediately placed on ice and processed within 1 h of collection. On alternate days of blood collection, urine and fecal grab samples were collected. Feces were sampled intrarectally while urine was harvested via sterile bladder catheterization. Fresh fecal samples were lyophilized and stored along with fresh urine samples at -20°C until analysis was performed. During recovery period, blood, fecal and urine samples were taken on d 1, 2, 3 and 14 to monitor response of mares after removal from treatments.

Table 1: Concentrations of ergot alkaloids in feedstuffs consumed by mares (DM basis)

Feed [a]	Ergovaline	Ergovalinine ppm	Total [b]
Hay	0.06	0.03	ND
E+	1.19	1.03	ND
E-	0.000	0.000	ND
Seed	ND	ND	3.500

[a] Hay = bermudagrass hay; E+ = sweet feed mixed with toxic endophyte-infected tall fescue seed and molasses; E- = sweet feed mixed with endophyte free tall fescue seed and molasses. Analyzed feeds did not contain GLMN. Seed = toxic endophyte-infected tall fescue seed prior to mixing with sweet feed, [b] Total = total ergot alkaloids; ND = not determined.

Laboratory analysis: *Feedstuffs.* Hay, E+ and E- were ground in a Thomas Wiley Mill (model 4, Thomas Scientific, Swedesboro, NJ) to pass a 2-mm screen followed by laboratory analysis for DM, ash, Crude Protein (CP), Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL)[13,14]. Dry matter of tall fescue seed was determined using lophylization, while DM of molasses and initial sweet feed was determined using a vacuum oven maintained at 60°C and 155.14 cm Hg for 45 h.

Total ergot alkaloids: Urine and feces were analyzed for gross ergot alkaloid concentration using a competitive ELISA described by Hill *et al.*[15-17]. Urine ergot alkaloids were expressed as ng ergot alkaloid per mg creatinine to correct for urine concentration and volume[16].

Ergovaline and ergovalinine: Feces, hay and mixed feeds (without GLMN added) were analyzed for ergovaline and ergovalinine using reverse phase Altima C18 column (3μ/50 mm L×4.6 mm ID) HPLC with an identical matrix guard column (4 mm L×3.0 mm ID). Samples (0.5 g) were saturated with 10 mL of 80% methanol and shaken for 2 h. After evaporation of methanol, samples were filtered and diluted in pure water (1:5) and 50 μL of sample was injected into HPLC. Solvents included, ammonium acetate and acetonitrile (injection vol 15 μL, flow 1.6 mL min^{-1}; run time 31 min).

Separation was accomplished on Water's 2695 HPLC separation module attached to fluorescence detector (474 module, Water's Corp., Milford MA). Sample elution occurred in gradient mobile phase with a 1.6 mL min^{-1}

flow rate for 31 min using solvent A: 5.39 g of ammonium acetate in 30% acetonitrile in line A, solvent B: acetonitrile in line B and water in line C. Acetonitrile was graded from 0 to 100% for 16 min. Excitation and detection wavelength were 255 nm and 435 nm, respectively.

Calibration curve was prepared from endophyte infected seed samples. Minimum analyte concentrations detected were 0.0128 and 0.024 ppm for ergovaline and ergovalinine, respectively. Limits of quantitation were determined to be 10 standard deviationxs from baseline noise of reagent blank and limits of detection was set as a 3:1 ratio of signal to noise. For validation, recovery was regressed against a calibration curve yielding $R^2 = 0.9976$ for concentrations between 0.0128 and 3.3 ppm ergovaline and $R^2 = 0.9783$ for concentrations between 0.024 to 3.104 ppm ergovalinine. Recovery for both analytes ranged from 95.92 to 99.8% when endophyte free sample were analyzed after spiking with endophyte infected seed samples of known concentrations.

Glucomannan adsorption of ergotamine: Glucomannan was evaluated for its ability to adsorb ergotamine. Water was added to GLMN (1 mL mg^{-1}) to from an aqueous solution simulating the intestinal environment. Ergotamines were incrementally added to solution (1 to 10 ppm) and extent of adsorption was determined by HPLC procedures previously described.

Hormones and metabolites: Plasma 3,4-Dihydroxy-phenylacetic Acid (DOPAC) concentrations were analyzed by HPLC with electrochemical detection using procedure proposed by Seegal *et al.*[18] as revised by Youngblood *et al.*[12]. Epinine was used as an internal standard to correct for DOPAC loss during sample processing and extraction. Serum cortisol was analyzed using Coat-A-Count RIA (Diagnostic Products Corporation; Los Angeles, CA) with an intra-assay CV of 5.84%. Prolactin was analyzed by RIA using protocol set forth by Colborn *et al.*[19] as revised by Fitzgerald and Davison[20].

Calculations and statistical analysis: Dry matter intake was calculated for hay, mixed feed and total diet by subtracting orts from initial feeds offered, after converting weights to a DM basis. Statistical analyses were conducted using MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Barn and barn by treatment interactions were analyzed as random effects. One barn included three replications per treatment the second only two replications per treatment, providing five replications per treatment. Repeated measures analysis was considered, resulting in no impact upon results. When significant ($p<0.05$), means were separated using Tukey's Honestly Significant Difference.

RESULTS

Nutrient composition was within acceptable ranges for all feedstuffs (Table 2). Dry matter intake of hay, mixed feed and total diet were uniform ($p = 0.91$) across treatments (% BW/d; Table 3). In the present trial evaluating non-gestating, non-lactating mares, serum PRL concentrations were not different ($p = 0.40$) among treatments (Fig. 1), which is in agreement with previous work[12]. Similarly, no differences ($p = 0.24$) were observed among treatments for plasma DOPAC (4.94 to 8.68 ng mL^{-1}; Fig. 2) or serum cortisol concentrations (4.48 to 5.69 μg dL^{-1}; Fig. 3). Further, rectal temperatures did not vary ($p = 0.70$; 37.15 to 37.34°C) among treatments of any phase (data not shown). Total fecal and urine ergot alkaloid excretion was greater ($p = 0.02$; Table 4) by mares consuming toxic endophyte-infected tall fescue seed during treatment phase (31.02 to 225.53 ng g^{-1} and 0.96 to 37.12 ng mg^{-1}, respectively). Similarly, fecal concentrations of ergovalinine and ergovaline followed the same trend with greater ($p = 0.054$) ergovaline (0 to 0.32 ppm) and greater ($p = 0.03$) ergovalinine (0.09 to 0.39 ppm) excretion by mares consuming toxic endophyte-infected fescue seed.

Table 2: Nutrient composition of feedstuffs containing endophyte-free and toxic endophyte-infected tall fescue seed consumed by mares (DM basis)

Feedstuff[a]	DM	NDF	ADF	ADL	Ash	CP
	----------	----------	%	----------	----------	----------
Hay	90.13	75.17	35.53	3.48	6.22	11.19
E+	84.30	32.67	13.54	ND	8.17	11.34
E-	85.14	32.94	13.75	ND	7.87	11.21
GLMN	91.93	56.74	51.78	ND	0.0049	28.10
Seed	85.00	ND	ND	ND	ND	ND
Sweet Feed	87.94	ND	ND	ND	ND	ND
Molasses	62.23	ND	ND	ND	ND	ND

[a]Hay = bermudagrass hay; E+ = sweet feed containing toxic endophyte-infected tall fescue seed and molasses; E- = sweet feed containing endophyte free tall fescue seed and molasses. GLMN = modified glucomannan derived from yeast cell wall. Analyzed feeds did not contain GLMN. ND = not determined.

Table 3: Effects of glucomannan (GLMN) upon dry matter intake of toxic endophyte-infected tall fescue seed by mares

		Dry Matter Intake[a]					
Item[b]	BW	Hay	Feed	Total	Hay	Feed	Total
		---------- kg d^{-1} ----------			---------- % BW d^{-1} ----------		
E-	500.24	5.93	4.25	10.18	1.17	0.83	2.00
E+	498.36	6.40	4.02	10.42	1.29	0.80	2.09
E+5	489.33	6.61	4.12	10.74	1.33	0.83	2.15
E+10	501.69	6.44	4.23	10.66	1.28	0.84	2.12
p-value	0.96	0.79	0.84	0.91	0.58	0.48	0.59
SE	17.743	0.501	0.203	0.616	0.098	0.018	0.104

[a]BW = body weight in kg; Hay = bermudagrass hay; Total = total DMI of hay and supplement, [b]Treatment diets: E- = bermudagrass hay and sweet feed mixed with endophyte free tall fescue seed and molasses; E+ = bermudagrass hay and sweet feed mixed with toxic endophyte-infected tall fescue seed and molasses; E+5 = bermudagrass hay and sweet feed mixed with toxic endophyte-infected tall fescue seed, molasses and 5 g GLMN twice daily; E+10 = bermudagrass hay and sweet feed mixed with toxic endophyte-infected tall fescue seed, molasses and 10 g GLMN twice daily. GLMN = modified glucomannan derived from yeast cell wall.

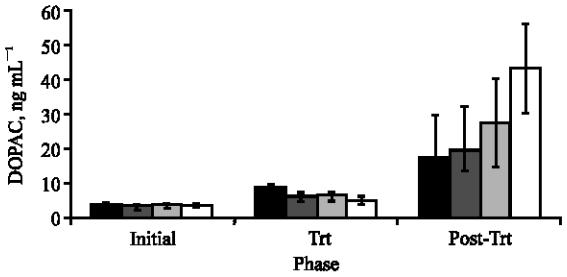

Fig. 1: Plasma DOPAC concentrations from mares before (1 d), during (12 d) and after (14 d) consumption of bermudagrass hay and sweet feed mixture containing endophyte-free tall fescue seed ( ) or toxic endophyte-free tall fescue seed ( ), or toxic ndophyte-infected tall fescue seed with 0 ( ), 5 ( ) or 10g ( ) glucomannan, an ergot alkaloid adsorbent, twice daily

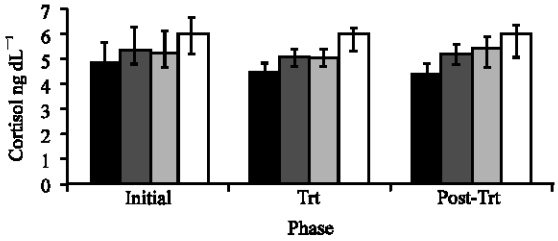

Fig. 2: Serum cortisol concentrations from mares before (1 d), during (12 d) and after (14 d) consumption of endophyte-free tall fescue seed ( ) or toxic endophyte-infected tall fescue seed with 0 ( ), 5 ( ), or 10 g ( ) glucomannan, an ergot alkaloid adsorbent, twice daily.

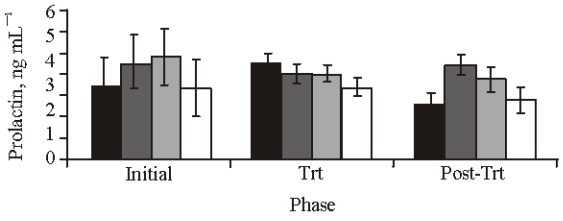

Fig. 3: Serum prolactin concentrations from mares before (1 d), during (12 d) and after (14 d) consumption of endophyte-free tall fescue seed ( ) or toxic endophyte-infected tall fescue seed with 0 ( ), 5 ( ), or 10 g ( ) glucomannan, an ergot alkaloid adsorbent, twice daily.

DISCUSSION

Feedstuff nutrient composition is listed in Table 2. Dry matter intake of hay, mixed feed and total diet were not affected (p = 0.91) by treatment (Table 3), indicating refusals did not significantly alter ergot alkaloid consumption, as confirmed by alkaloid excretion and physiological parameters. Akay *et al.*[8] however, reported an increase in DMI by steers consuming GLMN with or without exposure to ergot alkaloids. Similarly, Ely *et al.*[21] reported greater average daily gain and body condition score among cows and calves receiving GLMN while grazing toxic endophyte-infected tall fescue. Redmond *et al.*[22] found a tendency for toxic endophyte-infected tall fescue hay to reduce DMI and apparent digestibility of nutrients by mares whereas McCann *et al.*[23] found significant reductions in DMI and only a tendency to reduce apparent digestibility by yearling horses toxic endophyte-infected tall fescue hay. Despite this occurrence, complications resulting from consumption of ergot alkaloids by the mare are almost exclusively reproductive in nature, including thickened placentas, agalactia, embryonic loss and prolonged gestation[24]. Reports of reduced DMI of horses are less common than ruminants. Therefore, it would be unlikely to see a reduction in feed intake upon excessive consumption of toxic endophyte and data in the current study indicates that feed refusals were not severe, showing equal consumption of toxic endophyte-infected and endophyte-free tall fescue seed rations as well as bermudagrass hay.

The current project goal was to monitor fecal and urinary excretion of ergot alkaloids, as affected by GLMN, to potentially improve use of toxic endophyte-infected fescue pastures by horses. Fluctuations in blood hormones and metabolites were monitored as a secondary measurement of GLMN adsorption of ergot alkaloids. Ergot alkaloids have been shown acting as dopamine agonists contributing to ergot alkaloid-induced reductions in productivity[24-26] and reduction of circulating plasma DOPAC[27] and serum PRL in late-term pregnant mares[2,28,29].

Acting as dopamine agonist, ergot alkaloids bind to dopamine receptors, initiating a negative feedback mechanism that inhibits dopamine synthesis, thus reducing plasma DOPAC, a dopamine metabolite[27]. Over stimulation of dopamine receptors by ergot alkaloids is hypothesized to initiate an autoreceptor-mediated down-regulation resulting in a reduction in the total number of available dopamine receptors[30]. This down regulation of receptors serves as a control mechanism to reduce the likelihood of dopamine binding and signal transduction. Because of this interaction between ergot alkaloids and dopamine, plasma DOPAC was evaluated throughout the current trial as a determinant of ergot alkaloid excretion. However, no differences were observed among treatments for plasma DOPAC (p = 0.24; Fig. 2) or serum PRL (p = 0.40; Fig. 1) concentrations during treatment phase. During post-treatment phase, mares exhibited a similar response.

Table 4: Ergot alkaloid concentrations in feces and urine from mares before, during and after exposure to glucomannan, an ergot alkaloid adsorbent

	Treatments[a]					
Item[b]	E-	E+	E+5	E+10	p-values	SE
Total fecal Alkaloids, ng g^{-1} (DM basis)						
Initial	42.79	50.26	67.15	58.90	0.25	6.915
Trt	31.02[c]	203.87[d]	213.77[d]	225.53[d]	0.02	20.087
Post-Trt	260.92	270.26	265.86	221.23	0.33	18.147
Ergovaline, ppm (Air Dry basis)						
Initial	0.0	0.0	0.0	0.0	ND	ND
Trt	0.0[c]	0.28[d]	0.32[d]	0.30[d]	0.054	0.0509
Post-Trt	0.0[c]	0.31[d]	0.30[d]	0.29[d]	0.02	0.0377
Ergovalinine, ppm (Air Dry basis)						
Initial	0.0	0.0	0.0	0.017	0.5	0.0083
Trt	0.093[c]	0.38[d]	0.39[d]	0.36[d]	0.03	0.0393
Post-Trt	0.13[c]	0.43[d]	0.41[d]	0.47[d]	0.03	0.0544
Urine alkaloids	Total, ng mg^{-1} Creatinine					
Initial	0.13	0.10	0.29	0.032	0.54	0.118
Trt	0.96[c]	28.02[d]	35.47[d]	37.12[d]	0.005	2.878
Post-Trt	23.18	26.96	46.81	46.22	0.38	10.299

[a]Treatment diets: E- = bermudagrass hay and sweet feed mixed with endophyte free tall fescue seed and molasses; E+ = bermudagrass hay and sweet feed mixed with toxic endophyte-infected tall fescue seed and molasses; E+5 = bermudagrass hay and sweet feed mixed with toxic endophyte-infected tall fescue seed, molasses and 5 g GLMN twice daily; E+10 = bermudagrass hay and sweet feed mixed with toxic endophyte-infected tall fescue seed, molasses and 10 g GLMN twice daily. GLMN = modified glucomannan derived from yeast cell wall. ND = not determined, [b]Initial = pretreatment measurements (1 d); Trt = Measurements taken while exposed to treatment (12 d); Post-Trt = measurements taken after treatment removed (14 d). Total urine alkaloids expressed as ng ergot alkaloid/mg creatinine, [c,d]Within a row, means without a common superscript differ ($p<0.05$).

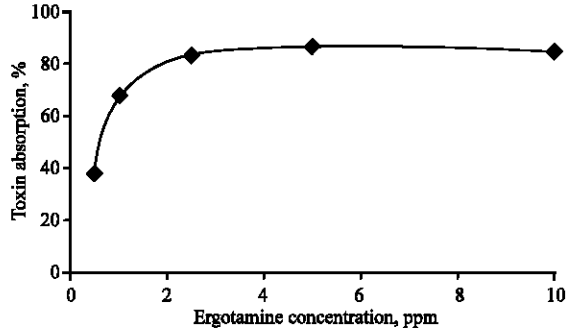

Fig. 4: *In vitro* kinetics of glucomannan adsorption of ergotamine in water (1 mg mL^{-1}). Reprinted with permission from Akay *et al.*[8].

Often, agalactia accompanies excessive consumption of ergot alkaloids and is due in part, to reductions in PRL secretions[24]. Unlike sheep and cattle, which often exhibit reductions in milk yield upon increased ergot alkaloid consumption[31,32], equine may experience complete agalactia[6]. Physiological status of pregnant and non-pregnant mares differ, with basal PRL concentrations lower in non-pregnant mares[23,28,33]. Therefore, it was not surprising that PRL concentrations did not differ in the current trial. Further, in the current trial, rectal temperatures did not vary ($p = 0.70$; 37.15 to 37.34°C) among treatments (data not shown), which is consistent with previous literature citing no change in rectal temperatures of mares consuming toxic-infected tall fescue[2,3,29], unlike ruminants, which often exhibit increased core body temperatures upon excessive ergot alkaloid consumption[8,24,34].

Secretion of cortisol in response to a stressor is well documented and includes exposure to ergot alkaloids[35-37]. Numerous researchers have reported cortisol concentrations analyzed from plasma, which ranged from 13 to 67 ng mL^{-1}[35,38,39]. In the current trial, using serum, cortisol concentrations ranged between 4.48 and 5.69 μg dL^{-1} (Fig. 3), similar to previous work analyzing serum (6.1 to 8.3 μg dL^{-1};[40]. In the present study, lack of change in serum cortisol concentrations upon feeding toxic endophyte-infected tall fescue seed further indicates that mares were not adversely affected by ergot alkaloids in the diet, regardless of GLMN consumption. This is supported by a numerical decrease in cortisol concentrations from treatment to the post-treatment phases. One explanation could be that non-pregnant mares have a greater threshold for stress than pregnant mares, which are under ever-increasing stress of gestation[41,42].

Supplemental GLMN was derived from inner cell wall of the yeast *Saccharomyces cerevisiae* (FEB-200; Alltech Inc., Nicholasville, KY) and modified to increase its adsorptive specificity for ergot alkaloids. As a result of this modification, an *in vitro* study revealed that GLMN had a concentration-dependent increase in affinity for ergotamines (Fig. 4). Ergotamines are among a specific class of ergot alkaloids called ergopeptines[43] and are readily available compounds structurally similar to ergovaline, a compound previously implicated with fescue toxicity[24,44,45]. In the current *in vitro* study, maximum adsorption was achieved when ergotamine concentrations exceeded 2 ppm, indicating a potential for adsorbing dietary ergot alkaloids and thereby reducing their metabolism and urinary excretion. In a confined feeding trial, supplementing 12 Holstein steers with GLMN, fecal excretion of ergovaline and ergovalinine was increased (0.9 μg g^{-1} (DM basis) and 0.3 μg g^{-1} (DM basis)) when steers did not receive GLMN[8]. Steers fed GLMN displayed greater DMI and reduced rectal temperatures, indicating glucomannan adsorption of ergot alkaloids sufficiently decreased alkaloid bioavailability within the gastrointestinal tract, therefore alleviating symptoms associated with ergot alkaloid-induced tall fescue toxicity[8].

Until the current project, efficacy of GLMN to alter ergot alkaloid excretion by equine had not been evaluated. Previously, a glucomannan variant, MTB-100 (Alltech, Inc., Nicholasville, KY), was reported to adsorb *Fusarium* mycotoxins in pigs, chickens and horses[9-11]. Although glucomannan used in the present trial, FEB-200, (Alltech, Inc., Nicholasville, KY) consists of the same glucomannan base as MTB-100, it has been specifically modified, by proprietary means, to increase its affinity for ergot alkaloid adsorption. In previous studies, feeding GLMN to cattle shifted ergot alkaloid excretion from urine to feces, a direct result of GLMN adsorption of ergot alkaloids[8]. A similar shift in ergot alkaloid excretion was not observed in the current trial regardless of GLMN supplementation. Although total ergot alkaloid excretion was greater in feces than urine, no change in urine concentrations were observed. Analysis of mixed feed revealed that only E+ contained ergot alkaloids, but analysis of hay offered to within both E+ and E- treatments confirmed the presence of ergovalinine and ergovaline. Bermudagrass hay likely contained a small percentage of dallisgrass (*Paspalum dilatatum* Poir.), a warm season forage containing *Claviceps paspali*, a fungus that produces paspaliterm mycotoxins[46-48]. Paspaliterms are lysergic acid derivatives[49] and therefore could inadvertently be included within the ELISA used to measure total ergots. This contamination may explain why ergot alkaloids were detected within feces and urine of individuals consuming E-. Further, there was an increase in ergot alkaloid excretion by all treatments during the post-treatment phase at which time, mares were grazing bermudagrass pasture that was later learned to have minor dallisgrass and tall fescue encroachment. During this post-treatment phase, mares did not receive grain supplements or GLMN; rather, this time was intended to monitor rebound in physiological parameters and ergot alkaloid excretion. These unanticipated sources of contamination do not appear to be confounding factors to the current trial given that hay and pasture contamination were minor and because they did not affect the ability to evaluate GLMN role in ergot alkaloid excretion. This is confirmed by greater ($p<0.05$) ergot alkaloid excretion by mares consuming E+ than E- during treatment and post-treatment periods. In addition, parameters for serum PRL[33,38,50,51], plasma DOPAC[12], serum cortisol[40,50,52,53] and rectal temperatures[54] were within normal physiological ranges for all treatments and were not influenced by ergot alkaloid content of the ration, hay, or pasture.

It is not clear what concentration of ergot alkaloids are needed to bridge the threshold required to initiate a physiological response in bovine or equine. However, physiological status of mares (i.e., gestating, lactating, or working) appears to have a direct effect upon ergot alkaloid concentrations required. For example, diets in the current trial were formulated in the same ratio (50 seed: 40 sweet feed: 10 molasses; as fed basis) as Youngblood *et al.*[12], except a different batch of seed was used, resulting in a greater ergot alkaloid concentrations (0.271 and 3.500 ppm/seed, respectively) in the current trial. In addition, Youngblood *et al.*[12] fed tall fescue hay containing 0.045 ppm ergot alkaloids. Therefore, mares in the current study received more ergot alkaloids than those fed by Youngblood *et al.*[12]. Despite this fact, ergot alkaloid consumption by mares studied by Youngblood *et al.*[12] altered plasma DOPAC and urinary ergot alkaloid concentrations. However, mares were in early stages of pregnancy (65 to 100 d) whereas the current trial evaluated open, non-lactating mares, emphasizing physiological differences that gestation ensues. At current rate and mode of ergot alkaloid administration (i.e., tall fescue seeds to open, non-lactating mares), ergot alkaloid metabolism was limited, as shown by reduced urinary ergot alkaloid concentrations. Aside from gestation status, the reason present urinary ergot alkaloid data differed from that of Youngblood *et al.*[12] is not entirely clear. Further, for reasons that are currently elusive, GLMN successfully adsorbed ergot alkaloids from the diet of cattle but not equine. Rate of passage through alimentary tract is often less in ruminants than horses and may be the primary limiting factor for GLMN adsorption of ergot alkaloids and alteration of ergot alkaloid excretion from urine to feces in horses.

As stated previously, concentrations of ergot alkaloids needed to induce adverse physiological affects is, as of yet, unknown. In the current trial, diets were similar to Youngblood *et al.*[12], but contained greater ergot alkaloid concentrations, yet resulting in a limited response. Spiers *et al.*[55] fed 16 times more ergot alkaloids to rats than is necessary to induce toxicity in cattle, citing larger metabolic body weight of rats. Although, the metabolic body size of equine is more similar to cattle, horses posses a digestive system not unlike the rat. Both rats and horses have a greater rate of passage than cattle, potentially requiring a greater alkaloid load by horses than cattle before symptoms can be expressed.

Previous success of GLMN when fed to cattle[8], involved average ergot alkaloid concentrations (0.44 and 0.5 ppm, respectively) and GLMN (0.2%) analogous to the present study. Further, when horses consumed a variant glucomannan (MTB-100) also at a rate of 0.2%, marginal success was observed in adsorption of *Fusarium* mycotoxins[9,10]. Despite these similarities and previous successes, GLMN did not effectively alter ergot alkaloid

excretion when consumed by open, non-lactating mares. Additional work is needed to determine if exposure time between ergot alkaloids and GLMN can increase fecal ergot alkaloid excretion in equine. This may be achieved by finding a way of evenly mixing GLMN within the diet by use of a feed mixer or by intermittent GLMN feeding. Previous success of GLMN may have been aided, in part, by use of a commercial feed mixer when preparing a TMR for cattle[8] or prior to pelleting a diet for equine (personal communication)[9,10]. In the present trial, GLMN was top dressed and lightly mixed with sweet feed and may not have been able to effectively blend with ingesta at time of ergot alkaloid availability, thereby reducing GLMN adsorptive capabilities. In order for GLMN to be successfully used to adsorb ergot alkaloids from the diet of equine, methodologies will have to be found that complement feeding habits as well as characteristics of the equine gastrointestinal tract.

CONCLUSION

GLMN has demonstrated promise in both *in vitro* and *in vivo* to adsorb ergot alkaloids and diminish bioavailability. However, additional work is needed in order to determine its merit in diet of horses consuming toxic endophyte-infected tall fescue. Although GLMN successfully increased fecal ergot alkaloid excretion in cattle, a similar response was not observed when fed to non-pregnant, non-lactating mares, under current experimental conditions. Functionality of the rumen serves to reduce rate of passage as well as particle size while mixing ingesta to a greater extent than can be achieved by the simple stomach of non-ruminant herbivores. Presumably, a means of GLMN delivery that complements equine foraging habits and digestive processes is needed for GLMN to work as designed.

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