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ANIMAL SCIENCE

Physiological effects of endophyte-infected perennial ryegrass straw on female camels in the Middle East

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Abstract

Recently, the United Arab Emirates has increased importation of perennial ryegrass (*Lolium perenne*) straw as part of the grass/hay ration for camels. Unfortunately, perennial ryegrass straw may be infected with the endophyte *Neotyphodium lolii* which produces lolitrem B, a toxic alkaloid responsible for the disease '*ryegrass staggers.*' A range-finding study was conducted using 24 non-pregnant female camels fed four doses (0, 1111, 1478 and 2273 ppb) of lolitrem B-containing perennial ryegrass straw over 56 days to establish a threshold of toxicity in camels so that perennial ryegrass straw can be safely fed as part of their dietary ration. Physiological parameters were evaluated. Neurological deficits were evaluated by videotape and scored according to an established scale for the neurological syndrome ryegrass staggers. Camels in the endophyte-infected groups developed varying degrees of ataxia. Brain edema, degenerative renal and hepatic lesions as well as Purkinje cell vacuolar degeneration were observed in camels receiving the highest dose. To avoid clinical disease in camels, endophyte-infected perennial ryegrass straw should be fed at 500 ppb or lower lolitrem B; straw at 1000 ppb or higher should be diluted to achieve the 500 ppb threshold of toxicity.

Key words: Camel, Lolitrem B, Perennial ryegrass, Ryegrass staggers

Introduction

Camels consume a varied diet, one of the components of which is grass/hay/straw. Recently, the United Arab Emirates has increased the importation of perennial ryegrass (*Lolium perenne*) straw from Italy, Spain, and the United States as part of the grass/hay/straw ration fed to camels in intensive farming system. Unfortunately, perennial ryegrass may be infected with the endophyte *Neotyphodium lolii* which produces lolitrem B, a toxic alkaloid responsible for eliciting the disease known as '*ryegrass staggers*' in domesticated cattle, sheep, horses, alpacas, and deer (Johnstone et al., 2012. Johnston and Mayhew, 2013; Blythe et al., 2007; Gallagher et al., 1982; Tor-Agbidye et al., 2001). 'Ryegrass staggers' is a condition in which animals consuming endophyte-infected perennial ryegrass containing lolitrem B develop stilted gait, ataxia, tremors, and hypersensitivity to external stimuli (Fisher et al., 2004; Gallagher et al., 1981). The molecular site of action of lolitrem B is on large conductance calcium-activated potassium channels (Dalziel et al., 2005), specifically, the ß-4 subunit which is responsible for modulating motor control and is associated with the ataxia observed upon ingestion of this toxin (Imlach et al., 2008). The lolitrems are unique amongst tremorgenic neurotoxins, however, in that they have a long duration of action, yet their neurotoxic effects are completely reversible in most cases (Gallagher et al., 1986; Evans and Gupta, 2012; Fisher et al., 2004; Miyazaki et al., 2007). As such, livestock affected with ryegrass staggers regain normal muscle response within 4-7 days after being removed from infected feed and appear otherwise unaffected. Clinical cases of ryegrass staggers have been observed in camelids such as alpacas; however, it is currently unknown what level of lolitrem B is needed to produce this disease in camels.

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Range-finding experiments examining the threshold of toxicity for lolitrem B in camels are needed so that perennial ryegrass grass/straw can be fed safely as part of the dietary ration to camels. Thus, our objective was to determine the patho-physiological effects of endophyte-infected perennial ryegrass straw on camels containing known amounts of lolitrem B, under the environmental conditions (i.e., high heat) of the Middle East.

Materials and Methods

Animals and diet

This study was approved by the Animal Research Ethics Committee at the United Arab Emirates University, under protocol number A4-13. Twenty-four non-pregnant female camels (*Camelus dromedaries*) of 5-7 years of age were purchased from the Al Ain Livestock Market. Animals were transported to the Faculty of Food and Agriculture Farm, United Arab Emirates University and were given standard camel feed with a salt mineral supplement for 14 days to adapt to the facilities and handling. Animals were then randomly divided into four groups (n=6/group) and fed one of the following diets containing perennial ryegrass straw over 56 days: control group A (0 ppb lolitrem B), group B with 1111 ppb, group C with 1478 ppb and group D with 2273 ppb. Lolitrem B-containing perennial ryegrass (PRG) straw was mixed with protein pelleted supplement to give minimal, balanced nutrition. Forage was provided twice daily (0800 and 1700) at 120% of the average intake for the previous 5 days, with feed refusals from the previous day weighed before feeding fresh forage. A trace mineralized salt mix was available free choice. Animals had continuous access to fresh water and forage throughout the experiment. One camel assigned to the control group A died unexpectedly during the second day of the two week conditioning period. The cause of death determined by necropsy was due to metabolic acidosis with a concurrent impacted bowl. The control group of camels then numbered five.

Nutrient composition of the feed

Dry matter, crude protein, acid detergent fiber (ADF), neutral detergent fiber (NDF) and organic matter were measured at the Animal Nutrition Laboratory of the United Arab Emirates University, according to methods certified by the National Forage Testing Association for the four PRG treatment groups (Table 1). The nutrients levels were consistent with the Arab Centre for the Studies of Arid Zones and Dry Lands requirements for camels.

Analyses of feed for lolitrem B

To establish the lolitrem B concentration for the four treatment groups in this study, plant material was ground in a Cyclotec 1093 sample mill and passed through a 0.5 mm screen (Hovermale and Craig, 2001). Three ml of a 2:1 methanol:chloroform (v/v) mixture was added to 0.2 g of sample, capped and rotated for 18-24 hours in the dark. Samples were then centrifuged at 2,000 x *g* for 10 minutes and 1.6 ml of supernatant was pulled off and dried under nitrogen at ambient temperature. One ml of dichloromethane (DCM) was added to the evaporated supernatant, capped and sonicated for 10 seconds, followed by 10 seconds of mixing; this procedure was repeated with an additional 1 ml of DCM. CUSIL 500 mg/6 ml SPE cartridges (United Chemical Technologies, Bristol PA) were loaded onto a positive pressure manifold and pre-conditioned with 2 ml DCM. The samples were loaded onto the SPE, followed by a 2 ml DCM wash. A 0.5 ml wash of elution solution (4:1 DCM: ACN (v/v)) was added to the cartridges and positive pressure applied. The sample was then eluted with 3.0 ml of elution solution and transferred to amber HPLC vials for analysis by HPLC-fluorescence. The fluorescence detector was set with an excitation wavelength of 268 nm and an emission wavelength of 440 nm. A Zorbax RX-SIL, 5 µ, 4.6 x 250 mm analytical column (Agilent Technologies, Santa Clara CA) was used in conjunction with a hand packed silica guard column, running an isocratic mobile phase (DCM:ACN:H_2O 4:1:0.02 (v/v)) at 0.5 ml/min for 15 minutes (Gallagher et al., 1981; Miyazaki et al., 2004).

Table 1. Nutrient composition of the feed.

Measurement	Diets				
	0 ppb	1111 ppb	1478 ppb	2273 ppb	Concentrate
Dry matter (%)	93.8	93.4	93.7	93.7	94.0
Crude protein (%)	5.0	6.0	6.7	6.6	12.0
ADF (%)	38.1	37.2	37.2	37.1	18.5
NDF (%)	64.2	64.7	65.2	64.3	33.0
Organic matter (%)	88.2	87.9	88.9	88.8	-

Physiological parameters

Each animal was weighed weekly using a livestock scale. Body skin and distal limb temperature were evaluated daily using an infrared thermometer. The feet were examined weekly for any possible lesions. Blood was collected weekly and processed to determine CBC, by the ADFCA Diagnostic Lab in Al Ain, using standardized procedures. The following hematologic parameters were measured: hemocrit (HCT), hemoglobin (HGB), red blood cells (RBCs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells(WBCs), neutrophils, lympocytes, monocytes, eosinophils and basophils. The following chemistry values were measured: copper (Cu), glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, alkaline phosphatase (ALP), creatine kinase (CK), alanine aminotransferase (ALT), asparate aminotransferase (AST), lactic dehydrogenase (LDH), gamma glutamyl transpeptidase (GGT), calcium, phosphorus, iron, sodium, potassium, chloride, and magnesium.

Monitoring Neurologic Disease

The primary disease condition seen in horses, sheep, cattle, deer, and alpacas being fed endophyte-infected perennial ryegrass is a neurological syndrome called "ryegrass staggers." (Reed et al., 2011; diMenna et al., 2012; Johnstone et al., 2011) The diagnostic criterion for this condition is determined by evaluation of the gait of an animal (Galey et al., 1991). Affected cattle, sheep, horses and alpacas exhibit an onset of muscle tremors, stiffness of gait during walking, base wide stance and gait progressing to clinical signs of ataxia or incoordination. The gait of each of the 24 camels in this study was videotaped prior to the start of the feeding trial and twice a week for the 56 days of the trial period. Subsequently, they were videotaped on 14 days and 41 days after being placed on endophyte-free straw to evaluate degree of recovery in affected animals. Neurological parameters that were evaluated were placement of limbs during stance and walking; presence of muscle tremors in limb, neck and head muscles; presence of hypermetria (exaggerated limb movement); circumduction of a limb on turning; muscle weakness; and ataxia (incoordination) with or without truncal sway. An unexpected finding of "lower lip droop" similar to a lower lip facial paralysis was observed in some camels and noted when present in any camel during its gait evaluation. The scoring of the camels' gait (Table 2) and the aforementioned parameters was done by a specialist in large animal neurology who was blinded to the dose of lolitrem B each animal was receiving. The videotape of each camel had the animal being led by a handler for approximately 20 meters in one direction, turned and then walked back to the starting point. The person doing the videotape then focused in on the major muscle groups of each side of the animal to observe the presence of any muscle tremors post walking. An evaluation of the clinical signs, or lack thereof, was noted for the videotape on each animal independently.

Pathology procedures

The progressive development of the pathological lesions associated with consumption of perennial ryegrass straw was studied by a pathologist in necropsy material from two camels in the high dose group (D) and two camels from the control group (A). Standard necropsy and histopathology procedures were used by the pathologists in the ADFCA Diagnostic Lab in Al Ain.

Table 2. Neurological scoring system used for diagnosis of ryegrass staggers[a].

Score	Clinical signs
0	No clinical signs
1	No resting tremors; low-intensity tremor and/or in coordination with handling; slight stiffness of gait and base wide placement of limbs while walking
2	No resting tremors; moderate-intensity tremors and/or in coordination with handling; marked stiffness of gait and base wide placement of limbs during walking and standing
3	Spontaneous low-intensity tremors at rest; moderate to severe tremors and/or in coordination with handling; marked stiffness of gait; truncal ataxia; difficulty in rising; weakness
4	Pronounced resting tremors and incoordination; convulsive tremors and severe in coordination with handling; extreme spastic gait (hypermetria); truncal ataxia and weakness; falling down into lateral recumbency with difficulty in rising.

[a]Modified from Galey et al. (1991).

Statistics

GraphPad Prism (La Jolla, CA USA) was used for development of graphs and statistical analyses. Physiological parameters and blood values were evaluated by two-way ANOVA, defining $p < 0.05$ as significant.

Results and Discussion

Camels have a rumen-like forestomach and depend, like ruminants, on microbial metabolism of their diet to provide usable products for growth and energy. Observations of abortions, reduced food intake, poor weight gain, lower pregnancy rates, decreased milk production, muscle weakness, tremors, and spasms in camels have become a concern of the camel farmers of the United Arab Emirates (personal observation). The possibility exists that three different fungal diseases of forages may play a role in causing these clinical conditions. These diseases could result from either ingestion of high endophyte infected perennial ryegrass, or high endophyte-infected tall fescue or ergot infected forage. With water becoming a scarce resource in the Middle East, the need for securing high-quality forage is becoming paramount to maintaining optimal herd health and production (Mooney, 2011). Going hand-in-hand with this, however, is the need to ensure that this forage is "safe to feed" throughout the life spectrum of the animals in question. Thus, we focused this initial project on the establishment of threshold of toxicity levels in non-pregnant female camels for lolitrem B.

Physiological parameters

Body weight was adversely affected by consumption of lolitrem B-containing perennial ryegrass straw over 56 days (Figure 1A). Camels fed a diet free of lolitrem B had a larger body weight gain (7.4%) than the other three groups (-2.5%, 0.5% and -8.8% for 1111 ppb, 1478 ppb and 2273 ppb, respectively). Two-way ANOVA was significant for group ($p < 0.001$) but not for date, or the interaction of group x date. However, Figure 1A shows a splitting apart of the four groups as time progresses, with the error bars beginning to become distinct between groups. It is likely that if the experiment were carried out for an additional time period, the weight gain average of the four groups would have continued to drift apart, making date and the group x date interaction significant. This is in contrast to a study on pregnant cows where pre- and post calving body weights were not affected by increasing lolitrem B concentrations (Fisher et al., 2004). The phenomena of decreased weight gain has been seen in numerous other studies of livestock consuming endophyte-infected grasses containing the alkaloid ergovaline, a condition called "summer slump" and is most commonly associated witht ergovaline in tall fescue (*Neotyphodium coenophialum*) (Peters et al., 1992). The perennial ryegrass straw used in this study did contain ergovaline at approximately one-third the level in ppb of lolitrem B.

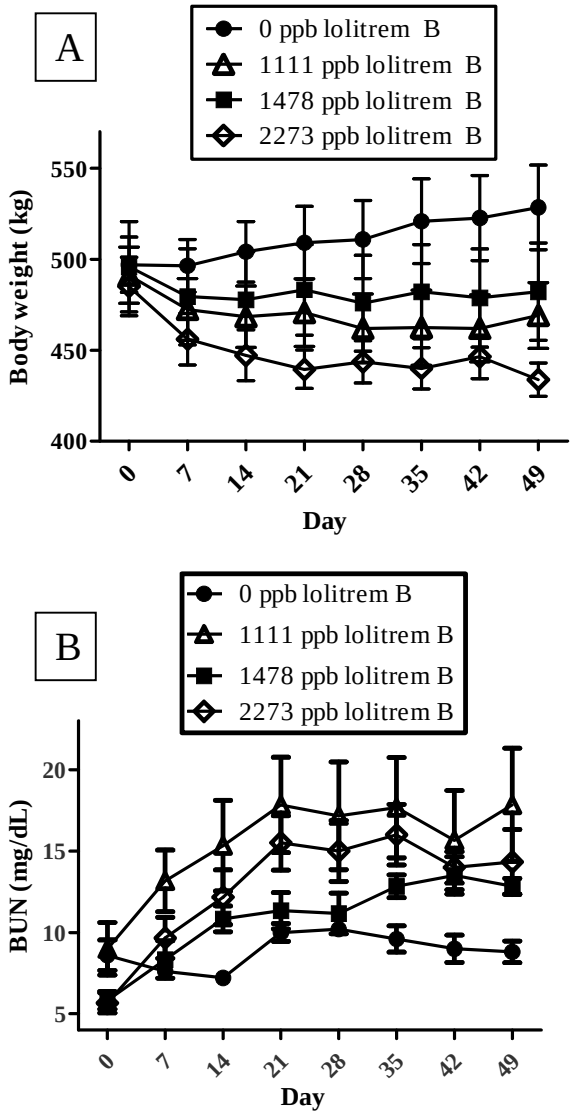

Figure 1. A and B. Body weight in female camels fed varying levels of lolitrem B-containing perennial ryegrass straw over 56 days.

Blood urea nitrogen (BUN) exhibited significant change for group ($p < 0.001$) and date ($p < 0.001$) but not the interaction of group x date by two-way ANOVA. Over the course of the study, the three treatment groups separated out and had higher levels of BUN than the control group

receiving 0 ppb lolitrem B (Figure 1B). Thus, the consumption of lolitrem B appears to increase BUN levels, which is indicative of kidney damage. This was supported by the gross and histopathological findings in the kidneys of both camels in the highest lolitrem B group (Group D) at necropsy.

All other physiological parameters in the blood showed no significant changes, as has been reported by Miyazaki et al. (2007). No dermatologic lesions were noted in any of the camels in this study except D5 and the loss of hair.

Neurological examination results

The mean and range of onset of clinical ataxia in all four groups of camels in this study was as follows: Group B (1111 ppb) had a mean onset of ataxia of 35.8 days +/- 17.0 (SD) (8.7-62.8 = 95% CI); Group C (1478 ppb) had a mean of 43.7 days +/- 11.6 (SD) (31.5-55.9 = 95% CI); and Group D (2273 ppb) had a mean of 19.8 days +/- 8.5 (SD) (10.9-28.8 = 95% CI). The camels in the control group (A) showed no signs of clinical ataxia throughout the study. Table 3 is highlighted with yellow color to illustrate which camels were ataxic in which time period of the study. It also includes details of the clinical evaluations of each camel videotape relative to a base wide stance or base wide limb placement during movement and/ or hypermetria of the rear limbs, weakness, and "lip droop". Weakness of voluntary movement and the presence of a "lip droop" were seen in a number of the camels in each group but rarely in the control camels over the 56 days of the study. Camels that develop the definitive sign of "ryegrass staggers" or clinical ataxia were graded from 1 to 4 and non-affected camels were given a zero. Development of a base wide stance or gait or hypermetria of the rear limbs were labeled Bw. These are seen in red in Table 3. These clinical signs often developed prior to ataxia or were seen intermittent in-between days where there was no clinical signs of ataxia. Each camel video was evaluated independently for that day with the evaluator not being aware of the dose of lolitrem B in the feed or the previous results for each animal. Weakness (w) was evident when the camels had difficulty rising, fell or had "truncal ataxia". The latter describes a sway of the body during forward movement and indicates a more severe ataxia.

At the end of the study, the A group was the control group with an mean of less than 100 ppb (designed as 0 ppb) which is the lower limits of the endophyte laboratory's measurement of lolitrem B; the B group had a mean of 1111 ppb lolitrem B; the C group had a mean of 1478 ppb lolitrem B, and the D group was the highest lolitrem B concentration with an approximate mean of 2273 ppb.

Camel D5 was the first one to start showing signs on day 10 and developed the most serious form of ryegrass staggers than continued until the completion of the study. In the last few weeks, the hair of this animal started falling out, a clinical sign seen only in this camel. While muscle tremors are the earliest clinical sign seen in horses, cattle, and sheep, this was not the case with camels eating moderate to high levels of lolitrem B in endophyte infected ryegrass. Camel D5 developed tremors in the pelvic limbs musculature on day 32 as did camel D15 and D16 on days 32 and 35 respectively. But these were somewhat rare occurrences and occurred late in the disease process rather than early.

By day 35, all six of the camels in the D group had or were showing clinical signs of ryegrass staggers. In contrast, in the C group, the earliest camel to become ataxic was C-3 at 16 days, but by the end of the study, the entire C group of camels had been affected. The B group with the lowest dose of lolitrem B had only 2 camels be affected early, i.e., by days 12 (B1) and 16 (B11) respectively. B1 showed fairly consistent signs of neurological dysfunction with either ataxia or a base wide stance/gait or hypermetria of the rear limbs throughout the study. B11 had onset of signs on day 16 with an intermittent return to normal gait in-between days of ataxia. This may have been related to the amount of feed this camel was eating each day or other unknown adaptive mechanisms. The differences may also be due to the method of evaluation in which each camel's video was viewed without reference to the previous day's results. The evaluator only noted what was evident in the short time of the video tape. By the end of the study, 4 camels in the B group had several days of being ataxic. An interesting note was a "dancing" motion exhibited by camel C17 during rest in pen for a short time during the first few days of the study.

Threshold levels are determined as that amount of toxin that will result in clinical signs. In this 56 day study, 2273 ppb lolitrem B affected all camels, 1478 ppb affected 2 camels between days 16 and 20 with all of the camels affected by day 54 to 56. With the lowest dose of 1111 ppb, 2 camels were particularly susceptible to the toxin at days 12(B1) and 16 (B11). At the end of 56 days, 4 of the 6 camels in the B group were affected. After 56 days, control camels A6 and A7 and two camels from the highest lolitrem B group, D5 and D16 were euthanized and sent to the ADFCA Diagnostic Lab in Al Ain for necropsy.

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Table 3. Summary of clinical signs seen in camels. The ataxia scores are highlighted in yellow and the basewide/hypermetria observations are highlighted in red.

Neurological evaluation
*Ataxia scores = 0-4; Bw = Base wide gait/hypermetria; * Lip droop: w = weak*

	Observation period in days of trial																			*Recovery days*	
	1-9	10	12	14	16	18	20	22	26	28	32	35	40	42	44	48	50	54	56	14	41
Control N=5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B1	0	0*	1*	1*	1*	1*	1*	1*	Bw*	Bw*	1*	0	Bw	1	Bw*	Bw*	0*	1*	1*	0*	0
B4	0	0	0*	0*	0*	0	0	0*	0*	0*	0*	0*	0*	1	Bw*	1*	0*	0*	0*	0*	0
B11	0	0	0	0	1	1	1	1	Bw	0	0	1	0	0	0	1	1	1w	1	0	0
B18	0	0*	0	0*	Bw	0*	0	0*	0*	0*	0	0*	0*	0*	Bw	1	1	0*	1	0	0
B19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Bw	0	0	0	0
B22	0	Bw	Bw	Bw	Bw	Bw	Bw*	Bw*	0 *	0	Bw*	1	1	1*	1	Bw	1*	1*	Bw*	Bw	Bw
C3	0	0	0	0	1*	1	1	Bw	0	0	1	0	Bw	0	Bw	2	2	2	3	0	0
C9	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Bw	1	1	1	Bw	Bw	0
C10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Bw	1	2	1	0
C17	0+	Bw	0	0	0	0	1	1	0	Bw	1	0	2	2	2	2*	1	2w	2	1	Bw
C20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Bw	2	Bw	Bw	1	0
C24	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1 *	2	0	0
D5	0	2*	2*	3*	3	3*	1w	3w	3	1*	1*	3w	3w	3*	3w*	4w*	3.5*	3*	3w*	Gone	Gone
D8	0	0	0	0	0	0	1	--	0	0	1	--	1	1	1w*	1w	1w	1	1	0	0
D12	0	Bw	Bw	0	0	0	1	1	1	1	1	1	2w	2w	1	Bw	1	1	1	1	0
D14	0	0	0	0	0	0	1	Bw	1	Bw	0	1	2	2	1	1w*	1	2	2	0	0
D15	0	0	0	0	Bw	0	0	Bw	Bw	Bw	1	2	2	2	1	2w	2w	2w	1	1	Bw
D16	0	0*	Bw*	1*	0	0	1	1*	1*	Bw*	1*	1	2	2*	1w*	2*	1	1*	1	Gone	Gone

+ dances in pen

Videotapes were also made of all the remaining camels during a recovery period on 14 and 41 days after they were placed on control feed with no lolitrem B. On day 14 of recovery, 9 of the remaining camels had normal gait and no sign of neurological deficits. Two camels, B22 and C9 had a base wide placement of limbs during walking and standing, and 5 camels were still grade 1 ataxic. By 41 days, no camels were ataxic and only three, B22, C17 and D15 had a residual base wide stance and gait. This recovery was best seen in the mildly affected camels. All of the camels on control feed (Group A) had no neurological deficits at any time during the study.

The "lip droop" seen most often in affected camels and only rarely in control camels appeared as if the lower lip had lost any tone and fell ninety degrees perpendicular to the lower jaw as if a partial facial nerve paralysis had occurred. This has not been reported in any ryegrass toxicosis study and this is the first report of ryegrass staggers in camels. During the recovery period, only 2 camels, B1 and B4 still had the "lip droop" evident on day 14 of recovery; it had resolved by day 41 of recovery.

Postmortem findings

On necropsy, the two camels from the 2273 ppb lolitrem B group (D5 and D16) showed gross lesions which is in contrast to what is reported in Jubb, Kennedy and Palmer (Maxie, 2007). This included a greenish colored liver compared to control camels (A6 and A7). See Figure 2. The kidneys showed adhesions between the renal cortex and the capsule with an outer layer of detached cortex (Figure 3). The adhesion of the capsule with the renal cortex was severe in one camel (D5) and slight in the other (D 16). The cut surface of the kidney revealed degenerative changes of the cortex and congested renal medullary blood vessels (Figure 2). The brain was edematous and blood vessels were congested (Figure 3). The gastrointestinal tract revealed a slight mucous inflammation. Internal organs of the control group camels were within normal limits. The weights of internal organs of both the control and affected camels, including the brain and divided by the camel weight at time of necropsy are in Table 4. On histopathology, the classic reported lesion of vacuolar degeneration of Purkinje cells in the cerebellum was present (Maxie, 2007). However, in the two affected camels, microscopic lesions were present in the liver and the kidney as well as the cerebellum (Figure 4). This is the first report of kidney dysfunction associated with lolitem B ingestion in camels. In a most recent report (Johnstone and Mayhew, 2013), horses fed 2000ppb lolitrem B in seed and hay developed reduced flow-mediated K+ secretion and had interference with aldosterone production or secretion. These abnormalities in the kidneys' ability to handle electrolytes in horses support this study's findings that lolitrem B at high levels can adversely affect kidney structure and function.

Table 4. The weights of the internal organs of control group camel (A6 and A7) and affected camels (D5 and D16) divided by body weight at the time of necropsy. The weights of the organs for the normal camels were within normal limits while some of the affected camels varied.

Organ / weight	Camel-1 (control)A-6 (gram/bwt kg=ratio)	Camel -2 (control) A-7 (gram/bwt kg = ratio	Camel-3 (treated) D-5 (gram/bwt kg = ratio)	Camel -4 (treated)D-16 (gram/bwt kg = ratio)
Liver	5552.3/438 = 12.7	6967.1/530 = 13.1	4758.1/437 = 10.9	5740.8/437 = 13.1
Left Kidney	547.2/438 = 1.3	678.3/530 = 1.3	712.1/437 = 1.6	577.1/437 = 1.3
Right Kidney	532.3/438 = 1.2	745.3/530 = 1.4	668.5/437 = 1.5	602.7/437 = 1.4
Brain	393.7/438 = 0.9	404.1/530 = 0.8	417.3/437 = 1.0	373.1/437 = 0.9
Age (years)	6-7	6-7	7-8	5-6

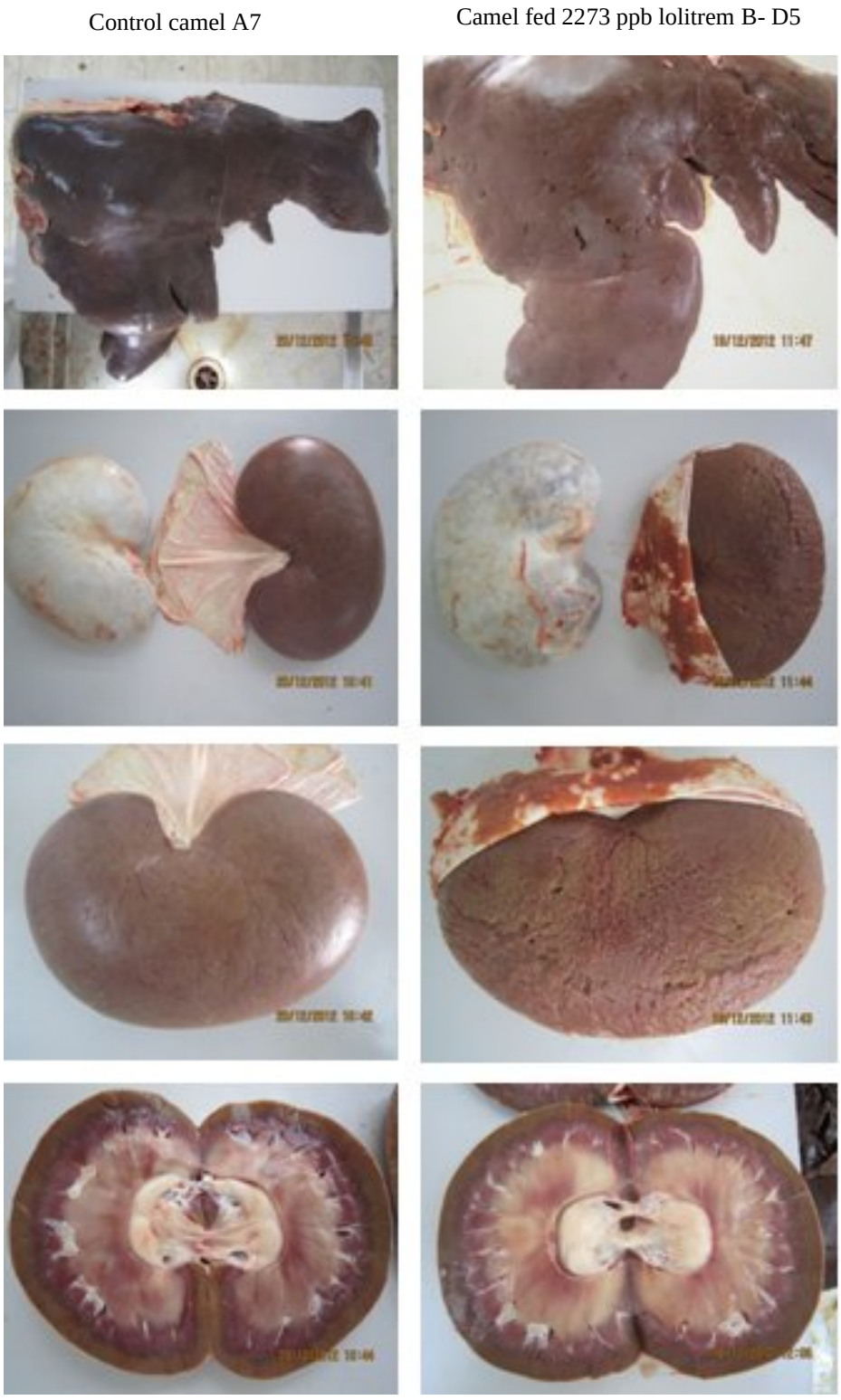

Figure 2. Pathologic examination of internal organs from camels fed perennial ryegrass straw containing 2273 ppb lolitrem B over 56 days.
(Top two photos show hepatic differences; bottom six photos illustrate renal abnormalities between a control animal and one receiving the highest dose of lolitrem B (D5)).

Figure 3. The brain of control camel A7 on the left and affected camel D5 on the right. Note, the edematous appearance of the brain on the right.

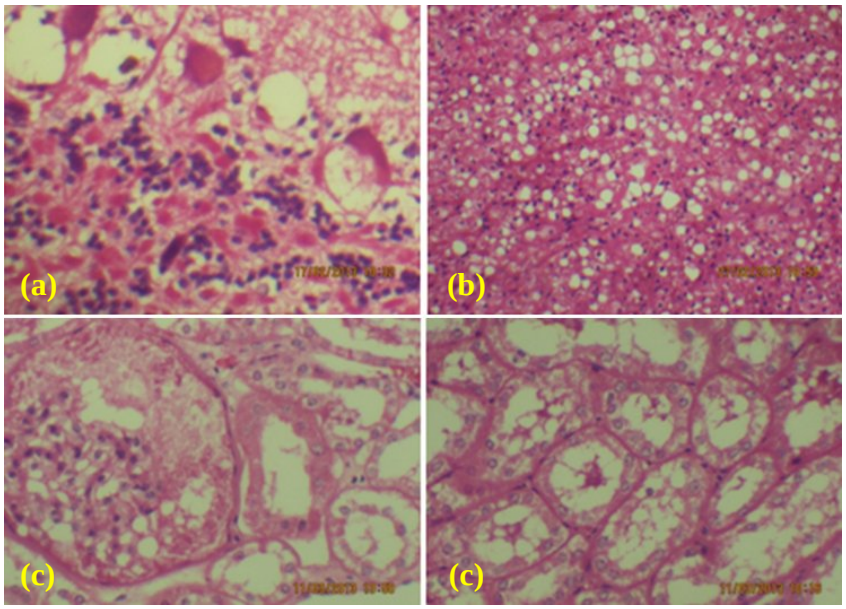

Figure 4 (a). An H & E stain of the gray matter of the cerebellum showing severe vacuolar degeneration of Purkinje cells in camel D5 (fatty necrosis). (b). An H & E stain of the liver of camel D5 showing severe vacuolar degeneration changes. (c). An H & E stain of the kidney of camel D5 showing severe vacuolar degenerative changes in epithelial lining of the convoluted tubules. The glomerular space and tubular lumens were obliterated with casts or proteinase material.

Conclusions

This is the first report on clinical ryegrass staggers in camels. Perennial ryegrass straw with 1000 ppb or greater caused a transient disease condition that resolved with return to "clean" feed. In this study, there was a reduction of clinical signs in 14 days in all camels and after 41 days, no clinical ataxia was evident. With this data over the time period of this study, one would need to say that camels are particularly sensitive to lolitrem B alkaloid. To avoid clinical disease, the authors suggest feeding endophyte infected perennial ryegrass straw at 500 ppb or lower and diluting all straw at 1000 ppb or higher. Imported feed should always be tested for this alkaloid.

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