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The effects of endophyte content on the nutritive value of drunken horse grass (*Achnatherum inebrians*) fed to sheep in Xinjiang province, China

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Abstract. Drunken horse grass (DHG) (*Achnatherum inebrians*) is a weed of the grazing lands of China, and contains an endophytic fungi (*Neotyphodium* spp.) producing the neurotoxin ergonovine. The following experiments investigate the effects of endophyte and ergonovine content on the nutritive value and on the symptoms of toxicity of DHG hay fed to sheep in Xinjiang province, PRC. Endophyte infection in DHG was controlled by spraying with Banner Turf during early growth (Low Endophyte), and infected DHG hays (High Endophyte) were prepared from nearby untreated swards. These hays were offered *ad libitum* to 3 groups of 4 sheep held in metabolism cages indoors over a 4-week period. In the first year (1996), a legume/grass (LG) hay was used as control and 200 g maize given to each sheep daily. In the second year (1996), a local grass (*Elymus siricibus*) was the control, and no maize was fed. Sheep given the LG hay consumed more DM (861 g/d) than did those given the treated (305 g/d) and untreated (264 g/d) DHG hays. Treated hay contained only 75 g/kg DM ergonovine compared with 200 g/kg DM ergonovine in untreated hay, and had higher *in vivo* digestibilities (61.3%) than untreated hays (54.8%). All sheep consuming DHG lost weight, and in the second experiment, without maize supplements, sheep were taken off treatments after 3 weeks as a consequence of excessive weight loss. There was a significant positive correlation between endophyte infection and ergonovine content of DHG hay, and significant negative correlation between DHG hay intake and its ergonovine concentration. An oral dose of 60 g ergonovine (2.3 g/kg live-weight) was sufficient to induce severe symptoms of toxicity which abated 11 hours after dosing. It was concluded that even low concentrations of ergonovine in DHG hay depress hay intake and the potential productivity of sheep consuming DHG hay.

Keywords. Drunken horse grass – *Achnatherium inebrians* – Endophytic fungi – *Neotyphodium* spp. – Nutritive value – Ergonovine toxicity – Sheep.

Effets de la teneur en endophyte sur la valeur alimentaire d'*Achnatherium inebrians *distribué au mouton dans la province de Xinjiang, Chine

Résumé. *Herbe enivrante des chevaux (DHG) (*Achnatherum inebrians*) est une mauvaise herbe qui pousse dans les parcours de la Chine, et renferme un champignon endophyte (*Neotyphodium *spp.) produisant la neurotoxine ergonovine. Des essais ont été conduits pour étudier les effets de la teneur en endophyte et en ergonovine sur la valeur alimentaire et les symptômes de toxicité du foin de DHG distribué au mouton dans la province de Xinjiang en Chine. La contamination du DHG par l'endophyte a été contrôlée par pulvérisation avec une bannière pendant le début de la croissance (faible endophyte), et les foins DHG infestés (endophyte élevé) ont été préparés à partir d'une pelouse non traitée et à proximité. Les foins ont été distribués à volonté à trois groupes de 4 moutons installés dans des cages à bilan pendant une période de 4 semaines. Pendant la première année (1996), un foin à base de mélange légumineuse/graminée (LG) utilisé comme témoin et 200 g de maïs ont été quotidiennement distribués à chaque animal. Pendant la deuxième année (1996), une herbe locale (*Elymus siricibus*) utilisée comme témoin a été distribuée seule (sans maïs). Le mouton recevant le foin LG a une ingestion de matière sèche plus élevée (861 g/jour) que le mouton recevant du foin DHG traité (305 g/jour) ou non traité (264 g/jour). Le foin traité renferme moins d'ergonovine (75 g/kg de matière sèche) et a une meilleure digestibilité* in vivo *(61,3%) que le foin non traité (respectivement 200 g/kg MS et 54,8%). Tous les moutons recevant DHG ont perdu du poids. Cette situation s'est aggravée dans le deuxième essai à cause*

d'une supplémentation par le maïs. Une corrélation positive et significative entre l'infection par l'endophyte et la teneur en ergonovine du foin DHG a été notée. En revanche, une corrélation négative entre l'ingestion du foin DHG et sa teneur en ergonovine a été démontrée. Une administration d'ergonovine par voie orale à une dose de 60 g (2,3 g/kg poids vif) a été suffisante pour induire des symptômes de toxicité sévères qui s'apaisent 11 heures après administration. En conclusion, il ressort que même de faibles concentrations d'ergonovine dans le foin de DHG réduisent l'ingestion du foin et le potentiel de productivité des ovins consommant le foin de DHG.

Mots-clés. *Herbe enivrante des chevaux* – Achnatherium inebrians – *Champignon endophytique* – Neotyphodium *spp. – Valeur nutritive – Intoxication par ergonovine – Mouton.*

I – Introduction

Considerable attention has been paid in recent years to the increasing prevalence of the perennial native grass *Achnatherum inebrians*, locally known as drunken horse grass (DHG), in the natural grasslands in the middle range of the Tianshan mountains in Xinjiang province of PRC. The grass has long been recognised as a weed because it is toxic to grazing animals. Livestock losses are not a significant problem since local animals rarely eat this grass when green. Poisoning usually occurs in the winter when forage supply is greatly limited or when animals are newly introduced to this pasture. Control of this grass in natural pastures can be achieved by the application of herbicide (Da *et al.*, 1988), cultivation (Fu and Ma, 1992) or by the introduction of competitive species (Li *et al.*, 1996), but these techniques are not a practical because the grass invades inaccessible remote, semi-arid and rocky mountain areas. An alternative approach is to isolate and remove the toxic principle in this grass, and develop new cultivars as species well adapted to these harsh grazing environments. It is now known that DHG contains an endophytic fungi (*Neotyphodium* spp.) (Li *et al.*, 2004) which is responsible for producing neurotoxins including ergonovine (Miles *et al.*, 1996). Our studies have shown that this endophyte can be controlled to some extent by systemic fungicides or completely removed by heat treating seed prior to establishment (Li, 1998). In a previous study, it was found that while sheep avoided grazing DHG of high endophyte (ergonovine) content, they would consume up to 40% of their diet as DHG apparently without affecting their health (Norton *et al.*, 2009). However there is no information available on the effects of endophyte content on the nutritive value of DHG, or any adequate description of the relationships between endophyte infection and ergonovine content of DHG, or of the levels of ergonovine required to produce observable symptoms of toxicity. This paper presents the results of feeding trials with sheep in metabolism cages given DHG containing different concentrations of ergonovine on voluntary intake, digestibility, live-weight gains and toxicity symptoms observed during a 4 week feeding period.

II – Experimental design and methods

Two experiments were conducted, the first experiment investigated the effects of endophyte content on the voluntary intake and digestibility of DHG hay fed to sheep in pens, and the second experiment studied the effects of administering a defined dose of ergonovine (in DHG) on the subsequent behaviour of sheep.

1. Experiment 1

In the first feeding experiment (1995), low endophyte (LE) DHG hay was made from fungicide (Banner Turf – active ingredient propiconazole 11 kg/ha) treated DHG pastures as described previously (Norton *et al.*, 2009), high endophyte (HE) DHG hay was made from untreated DHG pastures and a purchased legume grass hay was used as a control diet. Each diet was offered *ad libitum* to groups of 4 sheep held in metabolism cages in an enclosed room over a 4 week period, with additional supplements of 200 g corn seed per head per day. In the third week, hay intake and

diet digestibility were measured from the daily collection of faeces and urine from each sheep. In a second feeding experiment (1996), LE DHG was harvested from fungicide (Banner Turf) treated pastures, HE DHG was collected from similar untreated pastures growing nearby, and a common native grass (*Elymus sibiricus*) was also harvested and dried for feeding. These pastures were harvested as a younger age than in the previous year. Each diet was also offered to groups of 4 sheep in metabolism cages, but this time, without maize supplements. All hay was chaffed (3-5 cm) before feeding, and all sheep had free access to water and were given 2-3 g salt daily.

2. Experiment 2

Three healthy ewe weaners (Merino × Xinjiang fine wool), mean live-weight 26 kg, from the Nanshan farm flock were used, all sheep were grazing on dominant DHG pastures. Endophyte infected DHG was harvested from natural pasture, endophyte-free DHG was harvested from plots grown from heat-treated seed, and *Elymus sibiricus* (ES), a native grass not normally infected with endophyte was also collected from local grazing areas. All collected samples were field dried and ground to a fine powder, then dissolved in luke-warm water to make a slurry suitable for feeding to each sheep. The slurry was administered by manually placing small quantities in the mouth until swallowed, 350 g DM as HE DHG, LE DHG and endophyte free *E. sibiricus* was given to each sheep in this way over a period of one hour. All sheep were returned to a small paddock after treatment and observed closely over a 24 h period.

3. Analytical and statistical methods

DM content of feed and faeces were determined by drying samples at 80℃ for 24 h, followed by grinding to pass through a 1 mm sieve, and drying again at 105℃ overnight. N content was determined by the Kjeldahl digestion, steam distillation and titration of ammonia with 0.1 N HCl. *In vitro* digestibility was measured by incubation in pepsin-cellulase solutions (Goto and Minson, 1977). Ergonovine in feed was determined by the method of Miles *et al.* (1996). In the feeding experiments (Experiment 1) the significance of difference between treatments was determined by factorial analysis for a randomised complete block design. The significance of differences between treatments for feed intakes and live-weight changes were assessed by a Student's t test.

III – Results and discussion

In the 1995 experiment, fungicide treatment was effective in reducing but not eliminating endophyte infections in DHG, but did significantly decrease ergonovine content (Table 1). There were no significant effects of fungicide application on *in vitro* or *in vivo* digestibility, although when the partial digestibilities of DHG are calculated by removing the corn component of the diet (90% DM, 80% DM digestibility), sheep given the treated DHG has partial digestibilities of hay DM of 51.2% compared with a value of 39.4% from the untreated DHG hay.

Both treated and untreated hays were less palatable than the grass/legume hay, and despite the supplements of maize, these low hay intakes resulted in all sheep losing live-weight over the experimental period. In the 1996 experiment, fungicide treatment was more effective in reducing ergonovine contents of the DHG hay than in the earlier experiment, and at this time both the crude protein and *in vitro* DM digestibility of DHG was higher than that in the native grass. However, the untreated DHG proved to be highly unpalatable, and in the absence of maize supplements, sheep given DHG were not able to consume sufficient feed to maintain weight, and lost weight rapidly over experimental period. Apart from inappetence, no other clear symptoms of toxicity were observed. As a consequence, this trial was terminated at the end of week 3 and no measures of *in vivo* digestibility were collected. It was clear from comparing the two experiments that maize supplements provided some protection for these sheep from the toxic effects of ergonovine. Similar observations have been made for cattle consuming endophyte infected tall Fescue grass (Forcherio *et al.*, 1993).

Table 1. Effects of fungicide treatment of DHG hay on ergonovine and crude protein contents of hays, hay intake and digestibility and mean live weight changes in sheep offered the different diets

Diet fed	Ergonovine (mg/kg DM)	Crude protein (g/kg DM)	*In vitro* DM digestibility (%)	*In vivo* DM digestibility (%)	Voluntary Hay intake (g/d)	Live-weight change (g/d)
1995						
Grass/legume hay	0.8 a	88 a	48.7	59.1	854 a	82 a
Treated DHG	75 b	112 b	49.7	61.3	332 b	–64 b
Untreated DHG	200 c	113 b	48.3	54.8	294 b	–73 b
SEM†	24	2.6	2.01	5.3	42	7
1996						
E. sibiricus hay	1.5 a	83 a	40.9 a	ND	857 a	–9 a
Treated DHG	29 b	136 b	45.9 b	ND	472 b	–124 b
Untreated DHG	165 c	138 b	46.2 b	ND	147 c	–311 c
SEM†	5.4	2.2	0.93		25	22

†SEM, Standard error of the mean.
ND = not determined.
a, b, c: values within a column group with different letters differ significantly ($P < 0.05$).

There was a significant linear correlations between the level of endophyte infection, the concentrations of ergonovine in DHG DM and the voluntary intake of DHG DM by sheep offered these diets. These relationships are expressed by the following equations: EC = 2.8 + 3.73 EN (r^2 = 0.82) and DMI = 397 – 1.31 EC (r^2 = 0.85), where EC = ergonovine concentration in DHG (mg/kg DM), EN = Endophyte infection (%) and DMI = DM intake (g/sheep/d).

These relationships clearly show that ergonovine content increased with the level of endophyte infection and that there is a progressive decrease in voluntary feed consumption as the intake of ergonovine increases. It was also clear that supplements of maize significantly reduced the effects of ergonovine consumption on feed intake and live-weight changes in sheep, these supplements presumably increasing the rate of destruction of ergonovine in the rumen, thereby limiting the absorption and subsequent effects on metabolism. This observation suggests that supplementary feeding may be one way to decrease the toxicity of DHG for sheep.

In the second experiment, the ergonovine concentrations were 165 and 5 g/kg DM for the endophyte infected and endophyte free samples used for dosing the test sheep. This represented oral doses of about 60 and 1.5 g ergonovine per sheep. Sheep fed the native grass (*E. sibiricus*) and the low endophyte DHG resumed grazing an hour after dosing and apart from some slight diarrhoea in sheep given the low endophyte hay, no ill effects were seen during the 24 hours following dosing. However, the symptoms of toxicity appeared in the sheep dosed with the high endophyte DHG. In the first 3 hours after dosing, sheep looked weak and did not graze. At about 4 hours after dosing, the following symptoms were observed: shallow breathing accompanied by sudden deep inhalations, excessive salivation and foaming at the mouth, swaying and motionless stances for long periods of time, increased heart rate (120 beats/minute), occasional shivering of head and tail, incontinence, black and continuous diarrhoea after about 5 hours, unwilling to move with other sheep, but reacted when approached by humans. All symptoms had disappeared about 11 hours after dosing, and the sheep resumed grazing with the other sheep. These symptoms were similar to those previously observed in sheep grazing DHG and apparently suffering toxicosis (Yu, 1993), and to those observed in sheep grazing endophyte-infected rye grass pastures in New Zealand (Gallagher *et al.*, 1981; Siegel *et al.*, 1987). These symptoms are clearly related to ergonovine in the dose applied, and toxicity was induced by a dose of ergonovine representing 14.4 g DHG DM or 2.3 g ergonovine/kg live-weight. It is likely that the rapid administration of the dose may have induced more severe symptoms than if consumed naturally over a whole day.

However, Cao *et al.* (1991) and Wang and Dang (1991) found no symptoms of toxicity in sheep and goats given a dose of 20 g DHG DM/kg live-weight, while toxic symptoms could be induced in horses and donkeys given a lower dose (10 g DHG DM/kg live-weight). This observation is difficult to reconcile with those presented here because there is no information available on the ergonovine content of the DHG fed. It is likely that if ergonovine content declines with storage of dried DHG hay, and the doses administered to their sheep were much lower than in the present experiment.

It may be concluded from these studies that the toxic effects of DHG pastures are directly due to its ergonovine content, which is in turn, directly related to the extent to which DHG is infected with the endophytic fungus *Neotyphodium* spp. The principal effect of DHG consumption is to reduce the intake of DHG hay in proportion to its ergonovine content, with a consequence, sheep will lose weight rapidly and die if alternative source of feed or supplements are not offered. Even hays containing as little as 29 mg ergonovine/kg DM induced intake depression and weight loss.

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