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The grazing preferences of sheep on pastures containing drunken horse grass (*Achnatherum inebrians*) in Xinjiang province, China

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Abstract. Drunken horse grass (DHG) [*Achnatherum inebrians* (Hance) Keng] is a rapidly spreading perennial grass weed of western China. DHG contains an endophytic fungus (*Neotyphodium* spp.) which produces neurotoxins which cause morbidity in animals grazing these plants. The present study investigates the effects of fungicide (propiconizole) application, seed heat treatment, N and P fertiliser and water application on the growth and potential toxicity of DHG for grazing sheep in Xinjiang province of PRC. Two experiments are reported in which Merino/Xinjiang fine-wool sheep are grazed on pasture plots (5 × 10 m) of DHG and native grasses to which the above treatments were applied. Field application of fungicide did not effectively control DHG endophyte, reducing infection from 92 to 60%, and ergonovine concentrations from 358 to 273 mg/kg DM. Sheep selected other grasses in preference to DHG which formed on average 27% of DM eaten. The levels of endophyte in DHG did not affect preference, nor did the application of N and P fertiliser. In the second experiment, heat treatment of DHG seed prior to planting significantly reduced both endophyte (93 *vs* 5%) and ergonovine (308 *vs* 10 mg/kg DM) in DHG being grazed. Sheep consumed more DHG (54% removed, 61% in the diet) than did sheep grazing infected DHG pastures (38% removed, 51% in the diet). It was concluded that heat-treatment of seed is a cheap, practical and effective way of producing toxin-free DHG pastures in this area, and since neither plant survival nor responsiveness to fertiliser and irrigation was affected by endophyte presence, toxin-free DHG pastures may be a valuable new grazing resource for this low rainfall area. The present study also showed that while sheep will eat some DHG, they have a greater preference for other grasses, which increases as the proportion of other grasses in the sward increases.

Keywords. Drunken horse grass – *Achnatherum inebrians* – *Neoptyphodium* spp. – Ergonovine – Diet selection – Sheep.

Les préférences de pacage des ovins dans des parcours infestés par une mauvaise herbe* (Achnatherum inebrians) *dans la province de Xinjiang, Chine

Résumé. Achnatherum inebrians *(Hance) Keng (DHG) est une mauvaise herbe qui se propage rapidement en Chine de l'Ouest. DHG renferme un champignon endophytique (*Neotyphodium *spp.) qui produit des neurotoxines causant la morbidité chez les animaux consommant ces plantes. Cette étude évalue les effets de l'application d'un fongicide (propiconizole), du traitement à la chaleur des semences, de la fertilisation azotée et phosphatée, et de l'irrigation sur la croissance et le potentiel de toxicité du DHG pour des ovins sur parcours dans la province de Xinjiang de la Chine. Deux essais ont été conduits sur des moutons à laine fine placés sur des parties (5 × 10 m) du parcours de DHG et de mauvaises herbes naturelles qui ont été soumises aux traitements sus-indiqués. Le traitement du parcours par le fongicide n'a pas éliminé le DHG endophyte. En effet, il a réduit l'infestation de 92 à 60%, et les concentrations d'ergonovine de 358 à 273 mg/kg MS. Le mouton a sélectionné et a préféré d'autres herbes à la DHG qui représente en moyenne 27% de la matière sèche de la biomasse consommée. Les niveaux d'endophyte dans DHG n'ont pas affecté la préférence. Il en est de même pour la fertilisation azotée et phosphatée. Dans le deuxième essai, le traitement à la chaleur des semences de DHG avant semis a réduit l'endophyte (93 *vs* 5%) et l'ergonovine (308 *vs* 10 mg/kg MS) dans DHG pâturé. Les moutons ont consommé plus de DHG (54% prélevés, 61% dans la ration) que ceux pâturant dans un prcours infesté de DHG (38% prélevés, 51% dans la ration). En conclusion, il apparaît que le traitement à la chaleur des semences n'est pas coûteux et constitue une voie pratique et efficace pour l'obtention d'un pâturage de DHG* *dépourvu de toxines dans la zone d'étude. Par ailleurs, puisque la survie des plantes et la réponse aux fertilisants et à l'irrigation ont été affectées par la présence d'endophyte, les pâturages de DHG débarrassés de toxines peuvent être considérés comme étant une nouvelle et prometteuse ressource pastorale dans cette zone à faible pluviométrie. Cette étude a aussi montré que le mouton consommera une faible quantité de DHG mais aura une plus forte préférence pour d'autres plantes herbacées, qui augmentera avec l'augmentation de la proportion de ces plantes dans la pelouse.*

Mots-clés. *Herbe enivrante des chevaux –* Achnatherum inebrians – Neoptyphodium *spp. – Ergonovine – Sélection alimentaire – Mouton.*

I – Introduction

Achnatherum inebrians (Hance) Keng, commonly referred to as drunken horse grass (DHG), is a native perennial grass species found in the western Chinese province of Xinjiang. Although the toxic properties of DHG has know for many years (Hance, 1876), little research has been reported on its economic impact on grazing animals and the productivity of the natural grasslands through which it is rapidly spreading. This grass is ranked as one of the four major noxious pasture weeds of Xinjiang province (Da *et al*., 1988; Zhu and An, 1993). However serious losses of livestock are seldom a problem in DHG dominant pastures because locally adapted animals apparently avoid eating this grass. As a consequence, DHG is rapidly invading high quality native pastures, causing a serious degradation of these natural pastures. Past research has been focussed on the eradication of DHG from contaminated grazing lands.

DHG is infected by an endophytic fungus, originally described as *Acremomium* spp. (Bruel *et al.*, 1994), but more recently classified as *Neotyphodium* spp. (Li *et al.*, 2004, 2008) and it is likely that high levels of ergot alkaloids (ergonovine, Lysergic acid amide) are responsible for the toxic effects seen in grazing ruminants and horses (Miles *et al*., 1996). These endophyte infections in *Achnatherum* spp. may be effectively removed by the heat treatment of seed prior to planting, or controlled by the application of selective fungicides (Li, 1998). Removal of the toxic component allows the study of the specific effects of the endophyte and its alkaloids on environmental factors (soil fertility, water) affecting toxicity (alkaloid concentrations), the selectivity of sheep grazing DHG infested pastures and on the nutritive value of DHG in relation to its alkaloid content. The following paper reports the effects of endophyte (and ergonovine) content on the selection preferences of sheep grazing mixed swards of DHG and other native grasses.

II – Materials and methods

Two experiments were conducted in successive years at Nanshan Stud farm near Urumqi city in Xinjiang province PRC. Nanshan has a low rainfall (300 mm/year) mostly falling in summer, with daily temperatures ranging from 10 to 25℃ in summer to -5 to -20℃ in winter. Both experiments were conducted in areas of dominant DHG pastures on a sunny slope at an altitude of 1560 m.

Experiment 1: was carried out in 1995 and investigated the effects of fungicide application, and nitrogen (N) and phosphorus (P) fertiliser on the growth of DHG and on the grazing preferences of sheep for DHG in these pastures. The experiment was designed as a randomised complete block with a 2 × 2 × 2 factorial arrangement of treatments (fungicide F- or F+; N 0 or 100 kg N as urea/ha; P 0 or 100 kg P as superphosphate/ha; 3 replicates per treatment). Experimental plots (5 × 10 m with 1 m borders) were defined from an established area of pasture, and the systemic fungicide [Banner Turf© (active ingredient propiconazole 11 kg/ha)] was applied to half of the randomly selected plots on 19th June, then all plots were cut to 1 cm a week later, and after a further 2 weeks regrowth, the fungicide was applied again to ensure good control of endophyte infections. Single applications of N (as urea) and P (as superphosphate) were at the above rates to the appropriate plots on 24th July, and 12 week growing period was allowed before being grazed by sheep in mid-October. At this time, 6 ewe weaners (Merino/Xinjiang fine-wool) were grazed randomly across these plots over a 4 week period.

Experiment 2 (1996): because Banner Turf used in the previous year provided only partial control of endophytic infection, an alternative technique for endophyte control (heat treatment of DHG seed) was tested in this experiment. Dried seed from locally grown DHG was held at 37°C at 100% RH in a dessicator for 2 weeks, then air-dried and held at 4°C until sowing (E-). A portion of this seed was retained untreated to be used as a control (E+). This experiment was also designed as a randomised complete block with a 2 × 2 × 2 factorial arrangement of treatments [seed treatment E+ (control) or E- (heat treated); N 0 or 100 kg N as urea/ha; irrigation nil (W-) or 25 mm/ha every 10 d (W+); with 3 replicates per treatment]. The experimental site for this experiment was prepared by applying herbicide to the area following ploughing, and the treated and untreated DHG seed hand-sown (4 kg/ha) into each plot (4 × 5 m) on 24th May as determined by the experimental design. Irrigation was applied 17 days after sowing and at 10 day intervals thereafter. N was applied as a single treatment the day before irrigation was first applied. In the second experiment, six similar breed of sheep (7 months old) were grazed on these plots at the end of September, but grazing only lasted one week due to a shortage of available feed.

In both experiments, sheep were penned each night with *ad libitum* access to fresh water and salt, and were weighed at the beginning and end of the experimental period. Available dry matter (DM) on each plot was sampled (0.25 m^2/plot) at the start and total DM harvested at the end of each grazing period. The difference between total yields (kg DM/plot) at the beginning (IY) and the end of grazing (FY) was calculated as % removal [(IY-FY) * 100 / IY] and used as an index of grazing preference. DHG was sorted from other grasses, herbs and forbes (OG) at each harvest, and these values used to calculate % DHP selected in the diet [(IY_{DHG}-FY_{DHG}) * 100 / (IY-FY)]. Endophyte status was assessed by the methods of Clark *et al*. (1983) assigning a level of infection to plant material on the basis of the % blue tissue following staining with lacto-phenol cotton blue. Ergonovine content was determined by HPLC following solvent extraction of dried plant material. Results in each year were statistically analysed as separate factorial experiments.

III – Results

Table 1 shows mean values from Experiment 1. In this case, the fungicide used did not completely control endophyte infection, and the level of infection in both treated (60%, 273 mg ergonovine/kg) and untreated (90%, 358 mg ergonovine/kg) was high and likely to have remained toxic to grazing sheep if consumed in sufficient amounts. There were no significant differences in grazing preferences of sheep offered treated (43% removal) and untreated (34% removal) DHG, and these sheep preferred to graze other species to DHG. In this case, DHG represented only 24% of the forage selected when untreated, rising only slightly to 29% following a decrease in infection rate (and ergonovine content) with the application of fungicide. No symptoms of toxicity were observed in these sheep. The application of N and P fertilisers increased the yields of both DHG and the other grasses in the sward, but had no effect on the levels of endophyte infection in DHG or on the selective preferences of sheep for DHG.

In the second experiment, heat treatment of seed proved to be a very effective way of controlling endophyte infection, reducing endophyte infection from 94 to 3%. Table 2 shows that there was a significant reduction of endophyte infection in the DHG plants from 93 to 5% at first harvest, and this change resulted in a corresponding decrease in ergonovine concentrations from 308 to 10 mg/kg DHG DM. While yields of DHG were not affected by this decrease in infection rate, the preference of sheep for DHG increased significantly. The provision of both N and water increased the yields of DHG in the period studied, but did not affect the sheep's preference for endophyte free DHG over DHG with high contents of endophyte. There were no symptoms of toxicity observed in sheep grazing these pastures.

Table 1. Effects of fungicide (Propiconazole) application (kg active ingredient/ha), N and P fertiliser treatment (kg/ha) on DHG endophyte infection rates, ergonovine concentration, DM yield and selection (% removal) and intake of DHG by grazing sheep (Experiment 1)

Treatment	Infection (%)	Ergonovine (mg/kg DM)	DM yield (kg/ha)		% removal		% DHG selected
			DHG	Other grasses	DHG	Other grasses	
Fungicide							
0	92[a]	358	399	540	34	78	24.4
11	60[b]	273	374	512	43	77	29.0
Nitrogen							
0	77	350	301[a]	399[a]	33	78	24.2
100	75	280	472[b]	653[b]	44	78	29.0
Phosphorus							
0	73	309	363	432[a]	42	78	31.1
100	78	322	411	620[b]	38	77	24.6
SEM	5	38	33	57	4.7	2.6	2.6

[a,b]Values within a column with different subscripts differ significantly (P < 0.05).

Table 2. Effects of endopyhyte content (E- = heat-treated seed, E+ = untreated seed), N fertiliser (kg/ha) and irrigation on DHG endophyte infection rates, ergonovine concentration, pasture DM yields, selection (% removal) and intake of DHG by grazing sheep (Experiment 2)

Treatment	Infection (%)	Ergonovine (mg/kg DM)	DM yield (kg/ha)		% removal		% DHG in diet
			DHG	Other grasses	DHG	Other grasses	
Endophyte							
E+	93[a]	308[a]	163	71	38[a]	84	50.9[a]
E-	5[b]	10[b]	160	71	54[b]	79	60.6[b]
Nitrogen							
0	45	154	145[a]	67	45	74	56.8
100	49	164	177[b]	75	46	88	55.2
Irrigation							
0	52	167	113[a]	49[a]	44	82	55.3
25 mm/10 d	47	152	213[b]	94[b]	48	81	57.3
SEM	4	7	4.3	8.1	2.2	6.7	2.8

[a,b]Values within a column with different subscripts differ significantly (P < 0.05).

IV – Discussion and conclusions

Many native and improved grasses have now been recorded to contain endophytes (Moon *et al*., 2002; Wei *et al*., 2006) and the practical implications of these interactions are now only being realised. In the present study, the endophyte in DHG was most effectively controlled by heat treatment of seed, and while Banner Turf (100 times recommended dose) was the most effective of the foliar fungicides used, reducing ergonovine content by 62-83%, it has little value in the practical control of these endophytes in DHG. While N fertiliser application and irrigation increased DHG yields, there was no significant effect of these treatments on the level of endophyte infection in DHG. This observation suggests that edaphic factors are not related to the levels of endophyte infection, and that in general, improved management of these pastures will have little effect on their potential toxicity. It was expected from local observations that sheep would consume no DHG in their diet if offered other plants to graze. Table 3 summarises the observations made on the pasture intakes and selectivity of sheep for the different experiments, in both experiments, sheep consumed significant but limited amounts of DHG (71-107 g/d), usually consuming more DHG with low endophyte (and ergonovine) content that that of high content. The intakes of ergonovine varied from 61 mg/day in Experiment 1 down to 23 mg/day in Experiment 2, and since no toxic symptoms were observed in these sheep, it would seem that they are able to tolerate (detoxify) at least 60 mg ergonovine intake daily. This aspect is explored further in the following paper. It is concluded that while sheep in this study were able to detect and select against high endophyte DHG, they still consumed some DHG (70-107 g/d) to supplement their intakes even when feed availability was high.

Table 3. Mean values calculated for the intakes of sheep of DHG highly infected (E+) and poorly infected (E-) with endophypte infected (E+) and other grass species during each grazing period

Pasture species	Experiment 1		Experiment 2	
	DM intake (g/sheep/d)	Species intake as % total	DM Intake (g/sheep/d)	Species intake as % total
DHG (E+)	90	12	71	23
DHG (E-)	107	14	99	32
Other species	544	74	133	44
Total intake	741		303	

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