The tolerance of steers (*Bos taurus*) to sorghum ergot (*Claviceps africana*) in a feedlot during the cooler months in subtropical Queensland

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Abstract.   Two experiments tested the tolerance of steers (*Bos taurus*) to sorghum ergot (*Claviceps africana*) during cooler months in south-east Queensland. Sorghum grain containing 2.8% ergot and 28 mg/kg ergot alkaloids (84% dihydroergosine, 10% dihydroelymoclavine, 6% festuclavine) was incorporated into feedlot rations. In a previous study in summer–autumn, ergot (1.1–4.4 mg alkaloids/kg ration) severely reduced performance in steers when the temperature–humidity index (THI; dry bulb temperature °C + 0.36 dew-point temperature °C + 41.2) was ~70, whereas a THI of ~79 was tolerated by steers fed ergot-free rations. Experiment 1 was conducted in winter–spring, with rations containing 0, 2.8, 5.6, 8.2 or 11.2 mg ergot alkaloids/kg ration. All ergot inclusions depressed feed intake (14% average reduction) and growth rate (34% average reduction), even when the weekly average daily THI was less than 65. Rectal temperatures were occasionally elevated in ergot-fed steers ($P < 0.05$), primarily when the THI exceeded ~65. All ergot inclusions depressed plasma prolactin concentrations in steers. Experiment 2 was predominantly carried out in winter, with weekly average daily THI <65 throughout the experiment. Rations containing 0, 0.28, 0.55 or 1.1 mg ergot alkaloids/kg were fed for 4 weeks but produced no significant effect on feed intakes and growth rates of steers. Alkaloid concentrations were then changed to 0, 2.1, 4.3 and 1.1 mg/kg, respectively. Subsequently, feed intakes declined by 17.5% ($P < 0.05$), and growth rates by 28% ($P > 0.05$) in the group receiving 4.3 mg/kg alkaloid, compared with Controls. Plasma prolactin concentrations were depressed, relative to the Controls, by dietary alkaloid inclusion greater than 1.1 mg/kg, with alkaloid intake of 4.3 mg/kg causing the greatest reduction ($P < 0.05$). Cattle performance in these studies shows steers can tolerate up to ~2 mg ergot alkaloid/kg (0.2% ergot) in feedlot rations under low THI conditions (< ~60–65), but previous findings indicate a much lower threshold will apply at higher THI (>65).

Additional keywords: fungus, mycotoxin.

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Introduction

Sorghum ergot is a fungal parasite with the potential to affect a large proportion of sorghum crops worldwide. It was first reported in Australia in 1996 (Ryley *et al*. 1996) and subsequently identified as *Claviceps africana*, the species first characterised in Africa by Frederickson *et al*. (1991). This fungus produces several alkaloids, mainly the ergot alkaloids dihydroergosine (DHES), dihydroelymoclavine (DHEC) and festuclavine (FC) (Barrow *et al*. 1974). Infected sorghum grain usually contains a mixture of sphacelial and sclerotial tissues, collectively referred to here as 'ergot bodies' or 'ergot' (Blaney

et al. 2003, 2006). Several studies (Blaney *et al*. 2000*a*, 2000*b*, 2000*c*; Kopinski *et al*. 2007, 2008) have since shown sorghum ergot to produce the typical signs of toxicosis in pigs and cattle (impaired lactation, hyperthermia, reduced feed intakes) associated with rye ergot (*C. purpurea*) and millet ergot (*C. fusiformis*).

Sorghum grain is a very important component of feedlot rations in northern Australia, where high heat and humidity can already place a limitation on maximum feed intakes and growth. We have reported a previous experiment conducted during summer and autumn in south-eastern Queensland

where steers were fed rations containing 0, 1.1, 2.2 and 4.4 mg/kg of alkaloids (Blaney *et al.* 2011). All ergot-fed steers had hyperthermia, reduced feed intakes and growth depression when the temperature–humidity index (THI) was only 70, whereas a THI of ~79 was tolerated by steers fed an ergot-free ration. The corresponding concentrations of 'ergot bodies' in these rations were 0%, 0.25%, 0.5% and 1% (ergot content expressed as a percentage to align with industry standards). Given that the correlation between 'ergot' and alkaloids in different batches of infected sorghum is poor (Kopinski *et al.* 2008), it was concluded that ergot concentration should be limited to <0.1% in hot and humid conditions.

However, sorghum is grown in summer and mostly used in feedlots in winter and spring in southern Queensland, when the THI is usually moderate. Consequently, we conducted two more feedlot experiments over the cooler months of 1999 and 2000 to assess the tolerance of steers (*Bos taurus*) to sorghum ergot under such conditions.

Materials and methods

Animals, diets and experimental design

Two feedlot experiments (hereafter Expt 1 and Expt 2) were carried out at the Queensland Department of Primary Industries' Animal Husbandry Research Farm, Brisbane, Queensland, Australia, with the support and approval of the Animal Ethics Committee of the Queensland Department of Primary Industries (Approvals ARI/042/RES/DEC1998–1 and ARI/017/2000–1). The main objective of Expt 1 was to determine whether cattle had higher tolerance for sorghum ergot in feedlot rations during the cooler months of the year compared with the previously reported adverse effects under high temperature/humidity conditions (Blaney *et al.* 2011). Following this study, Expt 2 was set up to establish threshold tolerance for ergot in feedlot rations to provide guidelines for the Australian feedlot industry.

Expt 1

The experiment was carried out in Brisbane between 14 June (Day 0) and 29 November 1999 (Day 168). Thirty-five Hereford steers of initial liveweight 271.2 ± 15.85 ($\pm$s.d.) kg were used in a random block experimental design with seven replicates (individual steers) of five treatments. The steers were weighed full on Day 0, fasted (24 h without food, 15 h without water) overnight, re-weighed (Day 1; first day of feeding) and allocated to treatments and blocks by stratified randomisation on the basis of this fasted liveweight, and then randomly allocated to individual pens within these blocks. Pens were of two sizes, as described previously by Blaney *et al.* (2011), and steers from each treatment were evenly allocated across pen sizes. All pens had a minimum of 6 m^2 of shade.

The steers were adapted to a high-concentrate ration by incrementally increasing the ratio of grain-concentrate : hay from 1 : 9 to 9 : 1 between Days 1 and 15 (adaptation period). Grain free of ergot contamination (clean) was used during this period. Eventual feedlot rations were based on a combination of grain-concentrate and Rhodes grass (*Chloris gayana*) hay (9 : 1; air-dry). The grain-concentrate composition was (g/kg, air-dry): dry-rolled sorghum grain, 866; urea, 10; limestone, 12; cottonseed meal, 30; molasses, 56; bentonite, 20; ammonium

sulfate, 2; and pre-mix, 4. The pre-mix included (g/kg, air-dry) monoammonium phosphate, 455; salt, 237; trace elements/minerals (Zn, Fe, Cu, Mn, Co, K), 237; vitamin A/D3/E, 4; as well as Rumensin, 62 (22.5 mg monensin/kg total ration; Elanco, Eli Lilly Australia Pty Ltd, Sydney, NSW, Australia); and Eskalin, 5 (18 mg virginiamycin/kg ration; Phibro Animal Health Corp., Sydney, NSW, Australia). Treatments were formulated so that the rations had concentrations of 0 (Control; E0), 2.8 (E2.8), 5.6 (E5.6), 8.2 (E8.2) or 11.2 (E11.2) mg ergot alkaloid/kg, air-dry (average DM content 906 g/kg). The ergot alkaloid concentrations in the rations were incrementally increased from Day 15 so that designated treatment concentrations for groups E2.8, E5.6, E8.2 and E11.2 were achieved 3, 7, 11 and 14 days, respectively, after the end of the adaptation period. After 139 days (main experimental phase, including adaptation) the Control steers were weighed and removed from the experiment (Day 140; 1 November 1999) whereas other treatment groups were changed over to an ergot-free ration (post-ergot phase), the same as that previously fed to the Control group. They continued to be fed for a further 28 days before completion of the experiment (Day 168; 29 November 1999). As the original batch of clean sorghum grain was depleted in the main experimental phase a new batch was used for the post-ergot phase.

Expt 2

The experiment was carried out between 8 May (Day 0) and 11 September 2000 (Day 126) at the same site and in the same facilities as Expt 1. The design of the experiment, allocation of steers to treatments and pens, formulation of the basal ration and adaptation of steers to this basal ration (over 14 days) were similar to those described for Expt 1. Details specific to Expt 2 now follow. Thirty-six Hereford steers of initial (Day 0) liveweight 307.8 ± 12.04 ($\pm$s.d.) kg were used in a random block experimental design involving nine replicates of four treatments. Ergotised sorghum was introduced into the rations after the adaptation period (from Day 15) and rations were formulated initially so that they contained total ergot alkaloid concentrations of 0 (Control), 0.28, 0.55 and 1.10 mg/kg. However, due to the minimal effects of the ergot at these low concentrations early in the experiment, they were increased, incrementally, for some treatments from Day 43. Groups initially receiving (mg alkaloid/kg) 0 (E0) and 1.1 (E1.1) were not changed but the concentration of 0.28 was increased in two equal steps to attain 2.1 (hereafter E2.1) by Day 49, and of 0.55 was increased in four equal steps to attain 4.3 (hereafter E4.3) by Day 56. Thereafter these concentrations remained unchanged.

Procedures and sampling

Expt 1

Rations were fed once daily in the morning. The amount fed to each steer was adjusted on a daily basis so that some residue (~10% in excess of intake) remained in the bunk the next morning, thereby ensuring *ad libitum* intake. Feed residues were removed once weekly and weighed. Subsamples of feed and residue feed were dried in a fan-forced oven at 60°C for 48 h for DM determination, and proximate analysis was carried

out on the hay and grain concentrates subsampled weekly and bulked over 6-week periods.

The steers were weighed (unfasted) once weekly before the morning feeding. From the end of the adaptation period rectal temperatures were recorded once weekly at the time of weighing (0800–1000 hours) using a digital thermometer (Beiersdorf Australia Ltd, Sydney, NSW, Australia). Blood samples were taken from the tail or jugular vein of the steers just before feeding on Days 15 (just before feeding ergot), 36, 78, 140 (just before Control steers were removed) and 162. Plasma was recovered within 4 h and deep frozen at −20°C pending prolactin assay. Whole blood and plasma were also taken on Day 78 for extensive biochemical and haematological assays. The sampling technique, sample handling and analyses carried out have been described by Blaney *et al.* (2011). Steers were observed closely at least twice daily for signs of heat stress (panting, salivating, seeking shade and standing in water).

Expt 2

The general procedures and sampling regime were similar to those for Expt 1 except as follows. A proximate analysis of the feed and residue feed samples was carried out on samples bulked over 8-week periods. Blood samples were taken from the tail vein of the steers on Days 15 (just before feeding ergot), 36, 71 and 99, and treated as in Expt 1 for prolactin assay.

Environmental conditions

Climatic data from the local Bureau of Meteorology recording site on the station were used to define climatic conditions prevailing during the experiments. The THI was calculated on an hourly basis throughout the experiments using the same formula as in our previous study (Blaney *et al.* 2011), where THI = ta + 0.36dp + 41.2, where ta is dry-bulb temperature (°C) and dp is dew-point temperature (°C).

Source and alkaloid content of ergot in diets

The ergot-infected sorghum grain used in both experiments was from the same source as for our previous experiment (Blaney *et al.* 2011). It was stored in silos and withdrawn by auger as required. Estimates of ergot concentrations and alkaloid concentrations varied between samples, as shown below, but the ratios of different alkaloids remained relatively constant (84% DHES, 10% DHEC and 6% FC).

In Expt 1, alkaloid assays were conducted on grain samples taken on a daily basis (before dry-rolling the grain and ration formulation), bulked over each fortnight, mixed well, and subsampled with a riffle divider. A subsample (100 g) was used to estimate ergot content by visual separation and weighing, and a 1-kg sample was submitted for milling and alkaloid assay, using a high-performance liquid chromatograph method described in Blaney *et al.* (2003). Multiple assays (3–6) were conducted on each sample to improve precision of the estimate, and final figures were corrected for method extraction recoveries, as explained by Blaney *et al.* (2003). Over the experimental period, ergot concentrations in grain were estimated at ~28 g ergot bodies/kg (range 20–37; *n* = 8). Alkaloid concentrations in grain samples over the experimental

period averaged 27 mg/kg (24 mg DHES/kg), with a range of 21–31 mg/kg (*n* = 9).

In Expt 2, ergot was estimated at 27 g ergot bodies/kg (range 19–35; *n* = 4) in samples (200 g) of whole grain. Samples for alkaloid analysis were taken on a weekly basis after dry rolling the grain, in an attempt to reduce the observed variation in alkaloid content between samples and variation between replicate assay results. Over the experimental period, alkaloid concentrations averaged 28 mg/kg (23 mg DHES/kg), with a range of 22–31 mg/kg (*n* = 11).

Blood analyses

Prolactin concentrations in blood plasma were conducted using a radioimmunoassay as described by Downing *et al.* (1995), where the detection limit was 0.9 µg/L and where concentrations below this were given a value of 0.45 µg/L for statistical analysis. Methods for biochemical and haematological indices are given in Blaney *et al.* (2011).

Nutritional analyses and ration composition

The methods of proximate analysis of feeds and feed residues, and the method of estimating the metabolisable energy density of the diets (M/D) and their components have been described in Blaney *et al.* (2011).

Statistical analyses

The analysis of data involved a similar ANOVA approach to that described by Blaney *et al.* (2011) where the effects of dietary ergot alkaloid concentrations on rectal temperatures, feed intakes, growth rates and feed conversion were determined, with block and treatment as factors in the model. The time-series nature of the data was taken into account by an ANOVA of repeated-measures (Rowell and Walters 1976), via the AREPMEASURES procedure of GENSTAT (2015). This forms an approximate split-plot ANOVA (split for time). The Greenhouse–Geisser epsilon estimates the degree of temporal autocorrelation, and adjusts the probability levels for this. The prolactin data were transformed to the ln(concentration) scale before ANOVA in order to stabilise the variance. All analyses were done using GENSTAT (2015) with means compared using the protected least significant difference (l.s.d.) procedure operating at the 5% level of significance. The relationship between rectal temperature of the steers and THI was investigated using a range of regression models.

Results

Animal health and welfare

Expt 1

In the first 4 weeks several steers showed signs consistent with acidosis, specifically depressed food intake and diarrhoea, which often lasted for several days. Steers in all groups were generally slow to start eating and very low intakes were recorded in the first week of adaptation. Three steers were removed from the experiment: one suffering urinary calculi in the Control group (removed Day 92), one which stopped eating after 35 days in the E5.6 group (removed Day 36) and another from the same group that was injured in the pens (removed

Day 140). Some physical differences were observed between steers receiving ergot and those on the Control ration, particularly in relation to coat appearance. Steers were scored for coat length and roughness on Day 126 using a scale of: 1, sleek; 2, short (coat length); 3, medium; 4, medium-long; and 5, long. Average group scores were 1.2, 2.2, 3.0, 3.6 and 3.0 as dietary ergot increased. Thus, the coat of the steers fed ergot-containing rations tended to be longer and rougher than that of Control steers. A considerable number of the steers consuming ergot showed signs of heat stress during the second half of the experiment, including open-mouth breathing, panting, excessive salivation and high respiration rates. For instance, when assessed on Day 120, the numbers of steers showing some or all of these signs for the various treatments included: E0, 0 of 6 steers; E2.8, 4/7; E5.6, 3/6; E8.2, 7/7; and E11.2, 4/7. One steer from group E8.2 was often found standing with its front feet in the water trough. The apparent heat stress was exacerbated after exercise, as for instance during weekly weighing (50–100 m walk at 0800 hours). There were no clinical signs of gangrene, often associated with ergotism in cooler climates, in any of the experimental steers.

Expt 2

These steers showed few signs of ill health. However, the results from three steers were not used in the final analysis because the steers failed to adapt to the feeding situation and maintained very low intakes throughout the experiment, starting in the adaptation period. These included one steer in each of treatments E0 and E2.1, which were fed throughout, and one from E4.2, which was removed at Day 36. Another steer in the E2.1 group developed a large abscess on its leg during the last 2 weeks of the experiment, and its intake declined markedly. Data for this steer are included except for these last 2 weeks. The hyperthermic signs previously associated with ergot toxicosis, viz. excess salivation, high respiration rates, open-mouthed breathing, were uncommon in this experiment. Most steers maintained a long coat throughout and this did not seem to be related to treatment.

Ration composition

The composition of the rations is given in Table 1. In Expt 1, the ergot-infected grain tended to have higher crude protein (CP) and crude fibre (CF) but lower starch content than the clean grain but the estimated M/D was similar for both (Table 1). However, CP is based on total nitrogen concentration and becomes falsely high when fungal nitrogenous compounds are present, such as chitin (fungal cell wall, 7% nitrogen). In general, fungi utilise grain carbohydrate and lipids during invasion and increase the fibre content of infected grain (Williams *et al*. 1992). Consequently, there was a slight but consistent trend for CP

Table 1. Nutrient concentrations (g/kg DM) and estimated metabolisable energy density (M/D) in ration components and formulated grain concentrates

OM, organic matter; CP, crude protein; CF, crude fibre; EE, ether extract; Ca, calcium; P, phosphorus; –, not determined

	Period (weeks)	OM	CP	CF	Starch	EE	Ca	P	M/D (MJ/kg DM)
Expt 1									
Rhodes grass hay	All	899	50	–[A]	–	–	4.2	4.7	5.9
Clean sorghum[B]	1–20	986	115	31	682	33	0.2	2.4	13.9
	21–24	987	103	31	720	34	0.4	2.6	13.6
Ergot-sorghum	3–20	975	122	45	555	33	0.3	4.3	13.6
Grain-concentrates in rations[C]									
Ration E0	All	943	119	21	621	33	5.8	3.2	13.3
Ration E2.8	All	945	118	21	626	33	5.2	2.9	13.4
Ration E5.6	All	944	119	22	613	35	5.1	3.0	13.4
Ration E8.2	All	947	121	25	600	34	4.7	3.3	13.4
Ration E11.2	All	946	124	29	602	33	4.9	3.5	13.3
Expt 2									
Rhodes grass hay	All	896	61	–[A]	–	–	4.0	4.1	7.4
Clean sorghum	All	986	103	25	706	39	0.3	2.9	14.0
Ergot-sorghum	3–8	979	131	29	629	32	0.5	4.1	13.7
	9–18	977	129	30	643	32	0.7	4.4	13.7
Grain-concentrates in rations[C]									
Ration E0	1–18	939	109	24	629	28	6.4	3.4	13.2
Ration E1.1	3–18	946	109	30	605	30	5.4	3.2	13.3
Ration E2.1	3–7	942	110	27	625	29	8.4	3.3	13.2
	8–18	941	110	25	611	30	6.2	3.6	13.2
Ration E4.3	3–8	944	108	23	618	28	5.9	3.5	13.3
	9–18	942	111	19	597	31	5.6	3.3	13.3

[A]The neutral detergent fibre and acid detergent fibre concentrations (g/kg DM) in the hay were 736 and 437 in Expt 1 and 702 and 362 in Expt 2, respectively.
[B]Non-ergot contaminated sorghum, without additives. A new batch of sorghum was used in Weeks 21–24.
[C]Total grain-concentrate mix (without hay), following the adaptation period.

and CF contents to increase but starch content to decrease as ergot content in the grain-concentrates increased, but estimated M/D of the concentrates varied little between treatments. The Ca : P ratio in un-mixed sorghum averaged only ~ 0.10 but with the addition of limestone this increased to ~1.6 on average in the mixed grain concentrates (Table 1).

In Expt 2, ration composition was similar to that of Expt 1, with the ergot-infected sorghum having higher CP and CF content but lower starch content than the clean sorghum. However, at the lower inclusion rates of the infected grain in concentrates, the treatment differences were generally small for all three dietary components in the mixed grain-concentrates and differences in M/D were negligible (Table 1). The Rhodes grass hay was higher in quality than that used in Expt 1, having higher CP and lower neutral detergent fibre and acid detergent fibre content.

Climatic conditions and rectal temperatures

Expt 1

Rectal temperatures in steers, evaluated in relation to THI, are shown in Fig. 1. During the winter months the weekly average daily THI remained low (<65) and the number of hours per week that the THI exceeded 65 was also low (<60),

but both increased substantially during spring, especially from mid-October (Day 119) onwards. However, the weekly average daily THI did not exceed 70 in any week of the experiment. There was a significant treatment × time effect ($P < 0.01$) of ergot on rectal temperature of steers between Days 15 and 140 with significant treatment effects on Days 71, 120 and 134, the latter two coinciding with elevated THI, and trends on Days 99 ($P = 0.078$) and 127 ($P = 0.054$). Relative to the Control (E0 group), rectal temperatures were higher ($P < 0.05$): in all groups receiving ergot on Day 134; in all except the E2.8 group on Day 120; and in the E5.6 and E8.2 groups only on Day 71. Other differences between groups were not significant. In the last 4 weeks, when the Control treatment was discontinued, differences between the remaining treatments which were then fed ergot-free diets were not significant. However, in the absence of the Control group no assessment could be made on any carryover effects of ergot on rectal temperatures during this period.

Expt 2

Changes in the average daily THI across weeks and in associated rectal temperatures of the steers are shown in Fig. 2. With the experiment carried out mainly in the
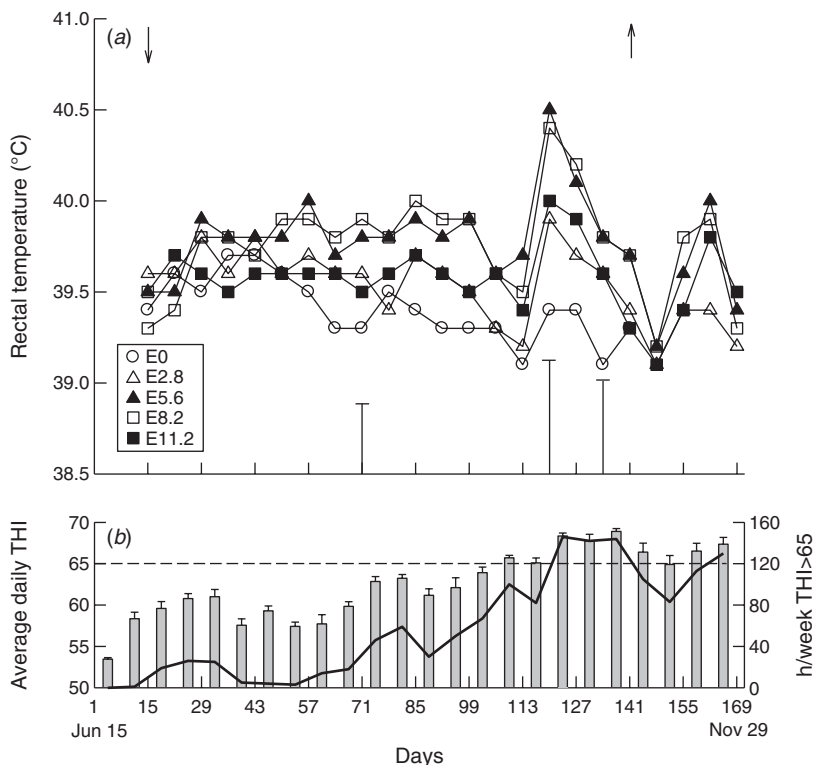
Fig. 1. Expt 1. (*a*) Changes in the rectal temperatures of Hereford steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloids (see text for details of treatments). Vertical bars represent the least significant differences ($P = 0.05$) for treatment means when the *F*-test was significant ($P < 0.05$). The arrows indicate when ergot was included (↓) or withdrawn (↑) from the diets. (*b*) Average daily temperature–humidity index (THI) for each week of the experiment. Vertical bars represent standard errors for average daily THI within each week. The superimposed line graph shows the number of hours each week when the THI exceeded 65.
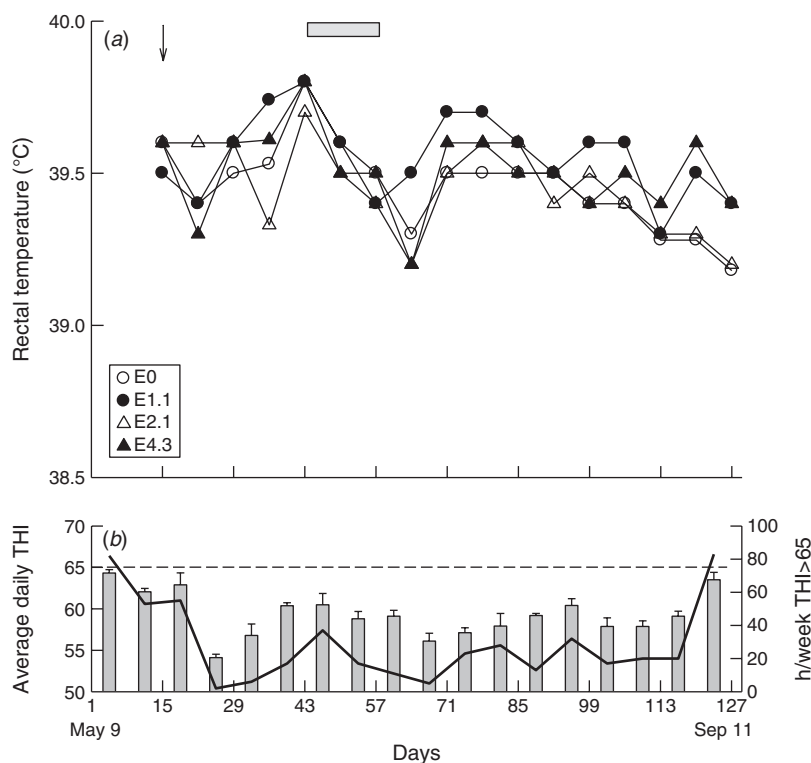
Fig. 2. Expt 2. (*a*) Changes in rectal temperatures of Hereford steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). The arrow indicates when ergot was included (↓) in the diets and the horizontal bar shows the period over which changes to alkaloid concentrations were made. (*b*) Average daily temperature–humidity index (THI) for each week of the experiment. Vertical bars represent standard errors for average daily THI within each week. The superimposed line graph shows the number of hours each week when the THI exceeded 65.

winter months, average daily THI remained below 65 throughout, and the number of hours per week that the THI exceeded 65 was less than 60 except in the first and last weeks of the study. There was no significant effect of ergot on rectal temperatures of the steers at any stage of the experiment with temperatures maintained ~39.5°C.

Animal growth, intake and food conversion

Expt 1

The liveweight changes are illustrated in Fig. 3 and summarised in Table 2. The liveweight change for the E0 group between Days 15 and 140 appeared relatively linear (average gain 1.35 kg/day) whereas those of the ergot-fed steers during this period appeared to occur in two stages (not analysed), higher initially and declining after an apparent change-point ~Day 78 (Fig. 3). The inclusion of ergot in the ration was associated with an average 34% reduction ($P < 0.05$) in growth rate of steers relative to the E0 steers from Day 15 to 140 but, within ergot groups, there was no effect of rate of inclusion in the ration during this period, during the 4 weeks after ergot was removed from the rations (Days 140–168) or for the combined periods ($P > 0.05$). The changes in intake are also shown in Fig. 3. Between Days 15 and 140, DM intake was

reduced by 14% on average with the inclusion of ergot in the diet, relative to the E0 group (see Table 2). Intakes by all ergot groups were lower than that of the E0 group over this period ($P < 0.05$). Furthermore, the E8.2 steers had lower intake than the E2.8 and E11.2 steers, which were not different, but not the E5.6 steers (see Table 2). There were no differences in intake between groups after the ergot was removed from the diets but trends over the total period (Days 15–168) were similar to those during the ergot-inclusion period for these treatments.

The feed conversion ratio (FCR; kg DM/kg growth) was higher ($P < 0.05$) for all ergot-fed groups except E11.2 relative to the Control group over Days 15–140 but within ergot-fed groups there was no effect of alkaloid concentration on FCR (Table 2).

Expt 2

The liveweight and intake changes are illustrated in Fig. 4 and overall effects are summarised in Table 2. Despite for the appearance of an effect of ergot on average daily gain in the second half of the experiment (see Fig. 4), differences between treatments were not significant either before or after the ergot concentration in the rations was changed. There was high between-animal variability, which apparently contributed to this effect. Intake was not affected by ergot inclusion in the
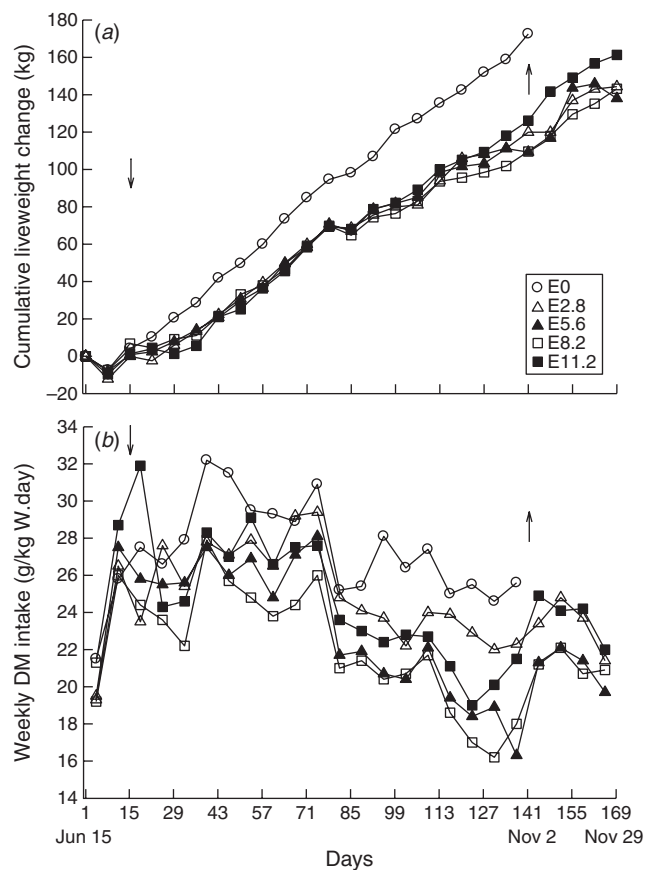
Fig. 3. Expt 1. (*a*) Changes in liveweight and (*b*) weekly average feed dry matter intakes by steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). The arrows indicate when ergot was included (↓) or withdrawn (↑) from the ration.

ration in the first 42 days (Days 15–57) but in the second half of the experiment (Days 57–126) intake by the E4.3 group was less ($P < 0.05$) than that of all other groups, which were not different. This effect resulted in reduced intake by E4.3 relative to other groups except E1.1 over the total ergot-feeding period (Days 15–126). The intake and growth rate effects of ergot were not translated into significant effects on FCR during any phase of the experiment.

Plasma prolactin concentrations

Expt 1

The changes in plasma prolactin concentrations are illustrated in Fig. 5. Plasma prolactin concentration for the E0 group was ~11 µg/L initially and increased thereafter to stabilise in the range 25–42 µg/L. Relative to the E0 group, there was a pronounced depression in plasma prolactin concentrations at Day 36 for all groups receiving ergot ($P < 0.05$), with concentrations declining to less than 5 µg/L for these treatments. The effect was less ($P < 0.05$) for the E2.8 group than for other ergot treatments. Despite a recovery in concentrations in the ergot-treated groups sometime between Days 36 and 78, values remained significantly greater ($P < 0.05$) for the E0 group than

for other treatments except for E2.8 at Days 78 and 140. On Day 140 the E5.6 and E8.2 groups had significantly lower plasma prolactin concentrations than all other groups. Plasma prolactin concentrations appeared to recover rapidly after removal of the ergot from the ration on Day 140, and by Day 162 averaged 37 µg/L without significant differences between treatments.

Expt 2

The changes in plasma prolactin concentrations are shown in Fig. 6. The plasma prolactin concentration for the E0 steers increased steadily over the experiment from ~27 to ~67 µg/L. Average plasma prolactin concentrations of ergot-fed groups trended less than Controls, but were not significantly affected by ergot inclusion in rations at Day 36. At Days 71 and 99 there was a stepwise depression in concentration as ergot levels increased. At both sampling dates the plasma prolactin concentration was significantly higher for the E0 group than for all but the E1.1 group, which was in turn not different to the E2.1 group, but the E4.3 steers had lower concentrations than all other steers (all differences $P < 0.05$).

Blood biochemical and haematological assays

The biochemical and haematological parameters measured in the blood samples taken on Day 78 of Expt 1 (results not shown) showed no abnormalities. There was no evidence of the signs of mild dehydration seen in our previous summer experiment (Blaney *et al.* 2011).

Discussion

Hyperthermia, feed intakes and growth

The depressed pattern of growth of ergot-fed steers reflected that of reduced intakes associated with ergot (Figs 3, 4), although in Expt 1 feed utilisation (FCR) was also slightly impaired by ergot. Treatments ranked similarly for intake and liveweight change over the main experimental periods in both experiments (Table 2), although in Expt 2 the effects of ergot were only significant for intake. Despite liveweight rankings mimicking those of intake in Expt 2, the within-treatment variability prevented liveweight differences from achieving statistical significance, notwithstanding an average difference in growth rate of 0.37 kg/day between the E4.3 and E0 groups between Days 57 and 126. In turn, reduced feed intakes aligned with reduced plasma prolactin concentrations across experiments (Figs 5, 6), as displayed by a generally similar ranking of treatments for both measurements over the main experimental periods. However, feed intakes were apparently unrelated to changes in rectal temperatures over most of the periods of dietary ergot inclusion (Figs 1, 2). This shows that alkaloid concentrations of 2–4 mg/kg and greater can affect intake even when the THI is too low to significantly impact on rectal temperatures.

On cursory examination, the effects of ergot appear greater during Expt 1 carried out in the winter–spring than in Expt 2 carried out predominantly in winter. However, several factors other than climatic conditions may contribute to this effect. The concentrations of ergot were higher in Expt 1 and the duration of this experiment was longer than for the following experiment. Nonetheless, it is evident that the effects of ergot

Table 2.   Effect of the rate of ergot inclusion in a sorghum-based feedlot ration on the intake, liveweight (W), liveweight change (full) and feed conversion ratio (FCR; kg DM intake/kg liveweight gain) for Hereford steers

Periods exclude the initial adaptation phase on ergot-free grain (Days 1–15). Within rows, means followed by the same letter are not significantly different ($P > 0.05$); –, not tested

						l.s.d. ($P = 0.05$)
Expt 1						
Treatment	E0	E2.8	E5.6	E8.2	E11.2	
Period I (Days 15–140)						
Day 15 liveweight (kg)	272.7	270.7	269.5	277.0	276.0	–
Feed DM intake (g/kg W.day)	27.6a	25.2b	22.9bc	22.1c	24.6b	2.35
Liveweight gain (kg/day)	1.35a	0.96b	0.86b	0.82b	1.00b	0.181
FCR (kg/kg)	7.4a	8.7b	8.7b	8.8b	8.2ab	0.86
Day 140 liveweight (kg)	441.2	390.7	378.0	379.9	400.9	–
Period II (Days 140–168)						
Feed DM intake (g/kg W.day)	–	23.3	21.0	21.3	23.7	n.s.
Liveweight gain (kg/day)	–	0.87	0.94	1.20	1.25	n.s.
Periods I and II (Days 15–168)						
Feed DM intake (g/kg W.day)	–	24.8a	22.5bc	21.9c	24.5ab	2.02
Liveweight gain (kg/day)	–	0.94	0.87	0.89	1.05	n.s.
FCR (kg/kg)	–	9.0	8.5	8.3	8.2	n.s.
Day 168 liveweight (kg)	–	415.1	403.8	413.4	436.0	–
Expt 2						
Treatment	E0	E1.1	E2.1	E4.3		
Period I (Days 15–57)						
Day 15 liveweight (kg)	319.1	315.0	316.1	316.0	–	–
Feed DM intake (g/kg W.day)	30.1	27.8	28.8	27.7	–	n.s.
Liveweight gain (kg/day)	1.33	1.20	1.15	1.27	–	n.s.
FCR (kg/kg)	10.6	8.6	13.3	7.4	–	n.s.
Day 57 liveweight (kg)	374.3	365.2	364.4	369.9	–	–
Period II (Days 57–126)						
Feed DM intake (g/kg W.day)	27.5a	26.4a	26.4a	22.7b	–	2.63
Liveweight gain (kg/day)	1.32	1.30	1.25	0.95	–	n.s.
FCR (kg/kg)	9.1	9.1	9.4	10.5	–	n.s.
Periods I and II (Days 15–126)						
Feed DM intake (g/kg W.day)	28.5a	26.9ab	27.3a	24.6b	–	2.49
Liveweight gain (kg/day)	1.32	1.25	1.20	1.06	–	n.s.
FCR (kg/kg)	8.8	8.4	11.7	8.8	–	n.s.
Day 126 liveweight (kg)	466.8	455.2	452.4	434.7	–	–

were amplified during the warmer spring months of the second half of Expt 1 (Figs 1, 3). During these warmer months the weekly average daily THI exceeded 65 and rectal temperatures in ergot-fed steers were episodically significantly greater than those in Control steers. By contrast, in Expt 2, carried out predominantly in winter, there was no effect of ergot on either intake or growth rate in the first phase of the experiment when inclusion rates were low (alkaloid range 0.28–1.10 mg/kg) but a depression in intake occurred when the maximum alkaloid concentration was subsequently increased to 4.3 mg/kg. This effect in Expt 2 occurred independently of any changes in rectal temperatures, which remained within the normal range throughout, but the reduced intakes of the E4.3 steers, after the change in ergot inclusion rates, were accompanied by depressed plasma prolactin concentrations on sampling days within this period (Days 71 and 99). In this respect the present results contrast those of our previous study (Blaney *et al.* 2011) from the same site using similar cattle and similar sorghum-

based feedlot rations, but carried out in the hot summer–autumn months when weekly average daily THI exceeded 65 for 15 out of the 22 weeks of feeding. Rectal temperatures of steers were markedly elevated with ergot-alkaloid inclusion in the diet (range 1.1–4.4 mg/kg) in that study accompanied by depressed plasma prolactin concentrations and markedly reduced intake and growth rates.

Parallel studies in the literature pertaining to the consumption by cattle of ergovaline from perennial ryegrass (*Lolium perenne*) infected with *Neotyphodium lolii* (Easton *et al.* 1996), and tall fescue (*Festuca arundinaceae*) infected with *N. coenophialum* (Hemken *et al.* 1981), provide clear evidence of an interaction of ambient temperature with the deleterious effects of ergot alkaloid ingestion, in what Dearing (2013) termed 'temperature-dependent toxicity'. For instance, various studies have shown that, at ambient temperatures within the thermoneutral zone for cattle, the effects of ergot alkaloids in tall fescue were negligible or muted compared with the more pronounced reduction in
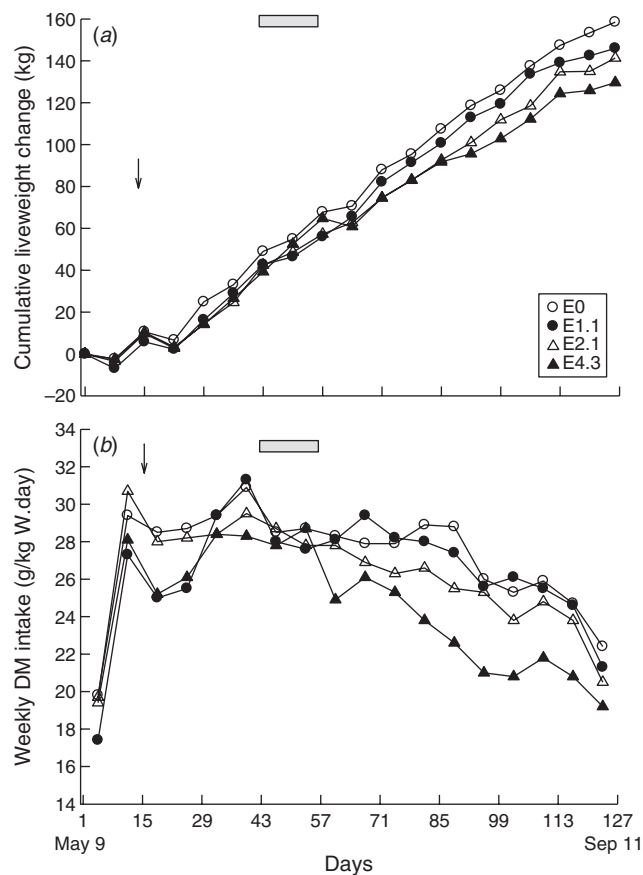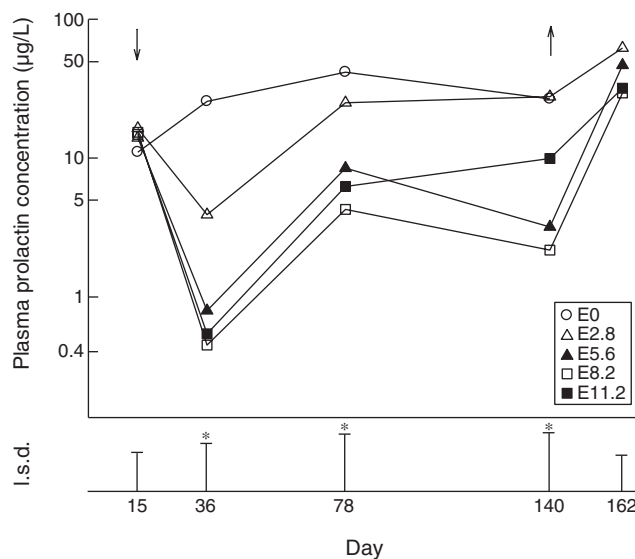
Fig. 4. Expt 2. (*a*) Changes in liveweight and (*b*) weekly average feed dry matter intakes by steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). The arrow indicates when ergot was included (↓) in the rations and the horizontal bar shows the period over which changes to alkaloid concentrations were made.

plasma prolactin concentration, increased rectal temperatures and depressed performance of steers fed these diets at temperatures greater than those of their thermoneutral zone (Hemken *et al.* 1981; Schmidt and Osborn 1993; Burke *et al.* 2001; Eisemann *et al.* 2014). These effects of ambient temperature on animal performance were often confounded with concomitant reductions in feed intake by ergot alkaloids, but in the study of Eisemann *et al.* (2014), the physiological effects were still observed when the intake effect was removed by restricting feed intake of steers to less than *ad libitum*.

Further compounding this effect is the finding that ergot-alkaloid intake appears to reduce the critical THI defining the upper limit of the thermoneutral zone for cattle (Blaney *et al.* 2011), thereby exposing cattle to toxic effects at lower than expected ambient temperatures. The results of Blaney *et al.* (2011) tentatively suggested a critical THI of ~70 for steers fed feedlot rations containing 1.1–4.4 mg alkaloid/kg, in summer. The present results suggest an even lower critical threshold THI as the weekly average daily THI did not exceed 70 in any week of either experiment, yet rectal temperatures were apparently affected by ergot alkaloids in the spring months of Expt 1 when the weekly average daily THI exceeded ~65. A reexamination of Fig. 1 in Blaney *et al.* (2011) provides some
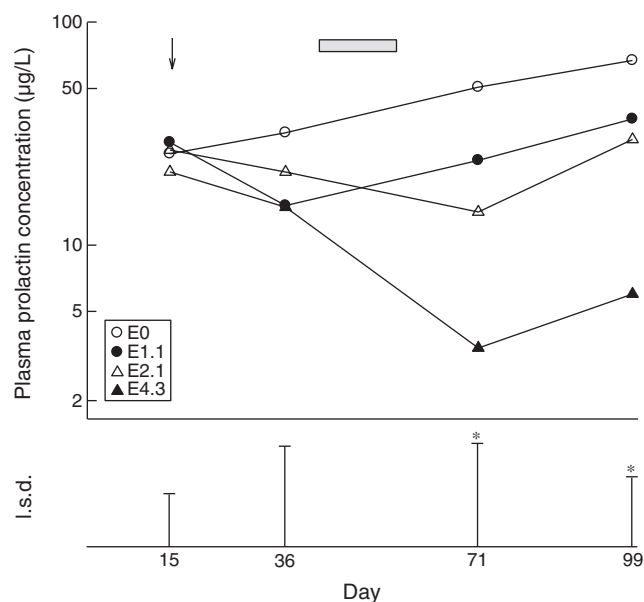

Fig. 5. Expt 1. Changes in the concentration of prolactin in the plasma of steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). Concentrations plotted are treatment means (geometric means) back-transformed after the ANOVA of ln (concentration). The arrows indicate when ergot was included (↓) or withdrawn (↑) from the ration. Separation of treatment means at each sampling date greater than the vertical dimension of the relevant least significant difference (l.s.d.) bar indicates significant difference ($P < 0.05$) where the *F*-test was significant (*; $P < 0.05$).

support for this revised value as the reported ergot-induced hyperthermia was not evident when average daily THI was below 65. It should be noted though that THI were averaged over the week whereas rectal temperatures were taken on a specific day and would thus be affected by the THI applying on that day of sampling, leading to occasional disconnects between the two measurements (e.g. Day 71 of Expt 1).

In Fig. 7 we present a closer scrutiny of this relationship between sorghum ergot alkaloid concentration in the diet, THI and rectal temperature of the steers using data from Expt 1, Expt 2 and Blaney *et al.* (2011). The combined data were initially fitted to an exponential model ($R^2 = 0.13$) and were then separated on dietary alkaloid concentration into groups of 0, ≤ 3 and >3 mg/kg, which improved the overall model fit ($R^2 = 0.19$). Subsequently, separate bent-stick models were fitted with further improvement in the relationships (combined $R^2 = 0.23$). According to these models, rectal temperature was constant at 39.43°C, 39.54°C and 39.66°C until the break-points at THI of 67.8, 66.6 and 66.9, whereupon it increased at 0.044, 0.071 and 0.062°C/unit THI, respectively, for the three alkaloid concentration groups described above. This analysis, although based on limited data especially at the higher THI, tentatively supports the earlier proposition of a THI-dependent expression of hyperthermia in sorghum ergot-fed cattle. However, refinement of critical THI, as indicated by break-points on the bent-stick models, will require more feeding scenarios given the high individual steer variability in susceptibility to ergot toxicosis displayed.

Apart from temperature and humidity as measured by the THI, lot-fed cattle can be exposed to additional heat stress from
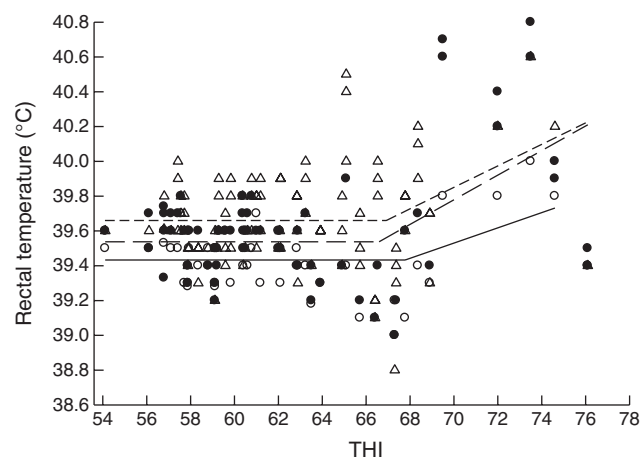
Fig. 6. Expt 2. Changes in the concentration of prolactin in the plasma of steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). Concentrations plotted are treatment means (geometric means) back-transformed after the ANOVA of ln (concentration). The arrow indicates when ergot was included (↓) in the ration and the horizontal bar shows the period over which changes to alkaloid concentrations were made. Separation of treatment means at each sampling date greater than the vertical dimension of the relevant least significant difference (l.s.d.) bar indicates significant difference ($P < 0.05$) where the F-test was significant (*, $P < 0.05$).

Fig. 7. The relationships (bent-stick fitted models) between the temperature–humidity index (THI), ergot alkaloid concentration in the feed and rectal temperature of Hereford steers given sorghum-based feedlot rations. Data are for individual steers/observation times from Expt 1, Expt 2 and Blaney *et al.* (2011) and are separated into diets without ergot (open circles, solid lines) and those receiving ≤3 mg/kg (closed circles, long-dashed lines) or >3 mg/kg ergot alkaloid (open triangles, short-dashed lines) in the ration.

direct solar radiation. This was only partly accounted for in our experiments, where all cattle had access to some shade. Wind speed and direction also can affect evaporative cooling, and Mader *et al.* (2006) have investigated these aspects in north American feedlots and proposed adjustments to the THI to better predict heat stress. Intense sunlight in the absence of shade, and low wind speeds to reduce evaporative cooling, could greatly exacerbate the effects of ergot, and apart from the production loss and welfare aspects, has the potential to cause high mortalities. Furthermore, Bourke *et al.* (2000) proposed that, quite apart from the effects of direct sunlight on the THI, it might interact with the pigments of ergot to exacerbate hyperthermic effects and mortalities in cattle denied access to shade.

Effect of sorghum ergot on prolactin and its consequences

Reduced plasma prolactin concentration is a very sensitive indicator of ergot alkaloid ingestion, considered to arise from modulation of dopamine D_2 receptors in the pituitary (Larson *et al.* 1995). The function of dopamine is to inhibit release of prolactin by lactotrope cells in the pituitary, and binding of ergot alkaloids to dopamine-2 receptors exacerbates this effect. Fletcher *et al.* (1997) found that prolactin concentration increased with ambient temperature in normal sheep, but not in those ingesting 1 mg/kg of ergovaline in pasture, and suggested

that prolactin has a role in temperature regulation. However, the other biogenic amines affected by ergot (nor-adrenaline and serotonin), which we did not measure, are more likely to be involved in the vasopressor effects contributing to hyperthermia and consequently to reduced feed intakes and growth.

In our previous summer experiment (Blaney *et al.* 2011), plasma prolactin concentration reduced from more than 70 µg/L to ~1 µg/L in steers ingesting rations containing 1.1 mg sorghum ergot-alkaloids/kg, and to <1 µg/L with 2.2 and 4.4 mg/kg. In the present Expt 1, prolactin concentration reduced from ~11 µg/L to ~4 µg/L in steers fed 2.8 mg alkaloid/kg, but then recovered, relative to the Control, whereas alkaloid levels of 5.6, 8.2 and 11.2 mg/kg reduced prolactin concentration to below the limit of detection for our assay, before also showing some recovery (Fig. 5). In the present Expt 2, prolactin was only slightly reduced from initial concentrations of ~27 µg/L by alkaloid concentrations of 0.28 and 0.55 mg/kg, but 2.1 and 4.3 mg/kg depressed prolactin concentrations to 14 and ~3 µg/L, respectively. It is noted that the average initial prolactin concentrations were much higher in the summer experiment than in the two winter experiments. Although statistical comparisons between these different groups of steers would be invalid, Schams and Reinhardt (1974) showed a positive correlation between prolactin concentration and the seasonal effects of daylength and temperature in growing cattle.

In summary, 1.1 mg alkaloid/kg in the diet caused severe prolactin depression in summer when the THI often exceeds 70 (Blaney *et al.* 2011), but 2.1 and 4.3 mg/kg seemed to have a lesser impact in winter (Expt 2) when the THI is usually <70. Apart from the different steers used in these experiments, the only other variable was the seasonal effect (daylength and THI),

which implies that higher THI might be exacerbating the effect of ergot in depressing prolactin release. This implies a more complex interaction between ergot alkaloid and dopamine receptor/prolactin release than a simple dose-effect relationship. Individual animal susceptibility and ability to recover from the effects of ergot-alkaloids obviously plays an important role, which appears most likely to account for the observation that in Expt 1, there was a greater reduction in mean plasma prolactin concentration in E5.6 and E8.2 steers compared with E11.2 steers at Day 140.

The rough hair coat of ergot-fed steers in Expt 1 is characteristic of cattle receiving ergot alkaloids from a variety of sources including rye ergot in grain (Dinnusson *et al.* 1971) and tall fescue (Schmidt and Osborn 1993; Nihsen *et al.* 2001; Schuenemann *et al.* 2005). Prolactin is one of the hormones regulating follicular cycling and its ergot-induced low concentration is thought to be involved in both disruption of the shedding of the winter hair coat and lack of inhibition of growth of the summer hair coat (Porter and Thompson 1992; Aiken *et al.* 2011). The implications are greatest when ambient temperatures are high as a long hair coat insulates an elevated body temperature, disrupts heat shedding and exacerbates hyperthermia in cattle given ergot-infected rations (McClanahan *et al.* 2008). Our present results, although relating mainly to steers fed in the cooler months, are nevertheless consistent with the general model of ergot-induced hyperthermia developed from the literature. The hyperthermia is a function of several inter-related factors such as high ambient temperature, lowered prolactin concentration, long hair coat and reduced upper critical THI, which conspire to depress cattle performance.

Regulation of sorghum ergot in grain for feedlots

Based on our experimental results and with the support of the feedlot industry, the maximum sorghum ergot inclusion rate in feedlot diets was set as 0.1% in 2008 under the sorghum trading standards of the National Agricultural Commodities Marketing Association (now Grain Trade Australia), and these standards have continued through subsequent seasons (Grain Trade Australia 2014). However, enforcing this limit at grain delivery points presents some challenges. After harvest, infected sorghum usually contains a mixture of sphacelial tissues and sclerotia (collectively referred to as 'ergot' or 'ergot bodies') with a highly variable alkaloid content (Kopinski *et al.* 2008). Mature sclerotia contain by far the most alkaloid (Blaney *et al.* 2003), but are hard to distinguish without expert input. Removal of ergot via gravity grading of grain has had limited success, because of the variable density of ergot bodies, and these ergot bodies are not evenly distributed in bulk grain.

The infected sorghum used in our feedlot studies was determined to have ~2.8% ergot content by weight, and 27–28 mg alkaloid/kg. Consequently, with this batch of grain, the 0.1% regulatory limit would translate to ~1 mg alkaloid/kg in grain, and ~0.8 mg/kg in a formulated ration. This appears a reasonable limit, but as Kopinski *et al.* (2008) have shown that occasional batches of infected sorghum with 0.1% 'sorghum ergot' might contain up to 1.6 mg alkaloid/kg, some additional caution is advised.

Conclusions

The tolerance of steers to sorghum ergot was shown to be dependent on two factors, viz. the concentration of ergot-alkaloid in the ration and the temperature–humidity conditions prevailing, as well as the interaction of these factors. Steers fed rations containing 1–2 mg ergot alkaloids/kg experienced only marginal effects on their performance when the THI was low (<65), but concentrations of 2–11 mg/kg produced severe depressions in feed intakes and growth, even when the THI was relatively low (<65), although the effect was more evident and associated with hyperthermia as the THI increased (>65). Sorghum grain contaminated with ergot at ~0.1% should only be fed in the cooler months, when the THI is moderate (<65), and if infection of crops becomes more frequent in future, then the percentage ergot should be supported by alkaloid assay (Blaney *et al.* 2003; Molloy *et al.* 2003).

Acknowledgements

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