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Effect of sorghum ergot (*Claviceps africana*) on the performance of steers (*Bos taurus*) in a feedlot

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Abstract. The effect of ergot (*Claviceps africana*) in naturally infected sorghum was assessed in feedlot rations. Thirty-two Hereford steers (*Bos taurus*) in individual pens with access to shade were adapted to feedlot conditions and then offered one of four rations containing 0, 4.4, 8.8 or 17.6 mg/kg of ergot alkaloids (84% dihydroergosine, 10% dihydroelymoclavine and 6% festuclavine), equivalent to ~0, 10, 20 or 40 g/kg ergot (sclerotia/sphacelia) in the rations. These rations were withdrawn at noon on the second day because of severe hyperthermia and almost complete feed refusal in ergot-fed steers. After recovery on ergot-free rations for 5 days, treatment groups were incrementally introduced, over a further 3–12 days, to rations containing 0, 1.1, 2.2 or 4.4 mg/kg of alkaloids (~0, 2.5, 5 or 10 g/kg ergot, respectively). Relative exposure to ergot was maintained, so that the zero- (control), low-, medium- and high-ergot groups remained so. Steers were individually fed *ad libitum*, and water was freely available. Steers in all ergot-fed groups had significantly elevated rectal temperatures at 0800–1000 hours, even when the temperature–humidity index was only moderate (~70), and displayed other signs of hyperthermia (increased respiration rate, mouth breathing, excessive salivation and urination), as the temperature–humidity index increased to 73–79 during the day. Plasma prolactin was significantly reduced in ergot-fed groups. Voluntary feed intakes (liveweight basis) of the ergot-fed groups were significantly reduced, averaging 94, 86 and 86%, respectively, of the feed intakes of the control group. Hair coats were rough. While the control steers grew from a mean initial liveweight of 275 kg to a suitable slaughter weight of 455 kg in 17 weeks (growth rate 1.45 kg/day), ergot-fed groups gained only 0.77–1.10 kg/day and took at least 5 weeks longer to reach the slaughter weight, despite removal of ergot at the same time as control steers were sent to slaughter. Sorghum ergot, even at low concentrations (1.1 mg alkaloids/kg feed) is severely detrimental to the performance of steers in the feedlot.

Additional keywords: fungus, mycotoxin.

Introduction

Ergot was first reported on Australian sorghum (*Sorghum bicolor* (L.) Moench) in early 1996 (Ryley *et al*. 1996), and later identified as *Claviceps africana* Frederickson, Mantle & de Milliano, the species first characterised in Africa (Frederickson *et al*. 1991). In contrast to known toxic ergot species such as rye ergot (*C. purpurea* Fr.Tul.), there were no reports of poisoning from sorghum ergot either in Africa or elsewhere (Mantle 1977), which had been ascribed to the 'weak pharmacological properties' (Mantle 1973) of the dihydro-alkaloids produced by *C. africana*, mainly dihydroergosine (DHES), dihydroelymoclavine (DHEC) and festuclavine (FC) (Barrow *et al*. 1974; Blaney *et al*. 2003).

Nevertheless, precautionary experiments were conducted and a trial where cattle grazed heavily ergot-infected sorghum (estimated at 0.4% ergot in the grain) for 28 days (average minimum–maximum temperatures, 5–22°C) produced no clinical signs of ergotism (hyperthermia, reduced growth, gangrene of extremities), but did show reduced blood prolactin concentrations (Blaney *et al*. 2000*a*). Reduced prolactin concentrations were also demonstrated in grower pigs fed high concentrations of sorghum ergot (Blaney *et al*. 2000*b*). Reduced prolactin concentrations arise from modulation of dopamine D_2 receptors in the pituitary, and are a very sensitive indicator of ergot alkaloid ingestion.

In 1997, an upper limit of 3000 mg/kg (0.3%) sorghum ergot in all stock food had been established in Queensland's Agricultural Standards Regulation 1997 (Anon. 2003), in contrast to the limit for rye ergot of 200 mg/kg (0.02%). Concern suddenly increased when cases of severely reduced milk production in sows and dairy cows occurred late in 1997 in Queensland and were shown to be caused by sorghum ergot (Blaney *et al*. 2000*c*). It thus became clear that sorghum ergot alkaloids had at least some of the detrimental effects associated with the alkaloids of rye ergot, and alkaloids produced by the endophyte *Neotyphodium lolii* Latch, Christensen & Samuels in perennial ryegrass (*Lolium perenne* L.), and *N. coenophialum* Morgan-Jones & Gams in tall fescue (*Lolium arundinaceum* Schreb.).

Subsequently, in 2003, several cattle feedlots in southern Queensland were reported to have suffered severe production

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losses (feed rejection and reduced growth rates) when cattle were fed sorghum grain later found to contain up to 30 g/kg (3%) ergot. As some comparison will become necessary, it is noted that rye ergot infects annual ryegrass (*L. rigidum* Gaudin) and perennial ryegrass in parts of southern Australia, and can cause severe hyperthermia in grazing livestock (Jessep *et al*. 1987), or in grain-fed ruminants if grain crops are contaminated with ergot bodies produced in the ryegrass (Peet *et al*. 1991; Bourke 2003). Australian rye ergot sclerotia contain mainly ergotamine, ergocryptine, ergocornine and their isomers (Blaney *et al*. 2009). Perennial ryegrass infected with *N. lolii* causes a complex syndrome that is a major source of livestock loss in south-eastern Australia in some seasons (Reed *et al*. 2005). Infected ryegrass contains ergovaline plus the non-ergot alkaloids lolitrems, peramine and paxilline. Hyperthermia due to ergovaline is involved, but the most obvious signs are 'ryegrass staggers' caused by lolitrem B, involving stiff limb gait and disorientation, progressing to involuntary body movements, muscle tremors and reluctance or inability to rise (Hovermale and Craig 2001). *N. coenophialum* also produces ergovaline and related alkaloids and causes major problems in the USA and other countries (Oliver 2005), but tall fescue pasture varieties grown in eastern Australia are free of the wild (toxic) endophyte.

There are no publications on the effect of sorghum ergot in cattle feedlot rations, other than our own preliminary reports of this work at conferences (Blaney *et al*. 2001; McLennan *et al*. 2001). We report here the details of an experiment demonstrating severely impaired performance of steers fed sorghum contaminated with ergot, under feedlot conditions.

Materials and methods

Experimental design

The trial was conducted from January to July 1998 in Brisbane, Australia. Hereford steers (*Bos taurus*) were transported from a commercial beef property near Roma in southern Queensland to the Rocklea Animal Husbandry Research Farm. After a 20-day period of acclimatisation on pasture, 32 steers were selected on temperament and liveweight for experimentation. These were used in a random block design involving eight replicates (individual steers) of the four treatments. The steers were allocated to treatment and to blocks by stratified randomisation on the basis of fasted liveweight (24 h without feed; 15 h without water), and then randomly allocated to individual pens within these blocks. Twenty large pens (~40 m^2) and 12 small pens (~15 m^2) were used, so that five steers from each treatment were housed in large pens and three in small pens. All pens had a minimum of 6 m^2 of shade. Water was freely available. Beginning on 28 January (Day 0), steers were gradually adapted to a high-concentrate ration (see 'Formulation of rations', below) by incrementally increasing the ratio of grain concentrate: roughage from 1 : 9 to 9 : 1 (w : w, as fed) over a 14-day period (Days 0–14).

On the first day of ergot-feeding (Day 15), steers were offered rations containing 0, 4.4, 8.8 or 17.6 mg/kg alkaloids (~0, 10, 20 or 40 g/kg ergot, respectively, as fed – see details below). Due to severe reaction (hyperthermia) of the steers as described in Results, the ergot-containing rations were replaced with ergot-free concentrate : roughage (7 : 3) for 2 days (Days 16–17), until steers had apparently recovered. A decision was then made to reduce the upper concentration of ergot to 4.4 mg alkaloids/kg total ration. Rations were again increased to 9 : 1 concentrate : roughage (Days 18–19). Beginning on Day 20, ergot-rich sorghum was gradually reintroduced into the rations by incrementally increasing the ergot-rich sorghum content every 2 or 3 days until the desired concentration was reached. The low-, medium- and high-ergot groups reached their target concentrations on Days 23, 26 and 32, respectively, of experimentation. During this process, the steers were kept in the same relative treatment groups as before; that is, the control group remained on the ergot-free ration (E0), the original 4.4 mg/kg alkaloid group received 1.1 mg/kg (E1.1), the 8.8 mg/kg group received 2.2 mg/kg (E2.2) and the 17.6 mg/kg group received 4.4 mg/kg (E4.4); the new rations contained ergot bodies (see below) at ~0, 2.5, 5 and 10 g/kg, respectively. These rations were fed until Day 118 when the control steers were sent to slaughter and the ergot-containing sorghum was removed from the other rations, and all remaining steers received the same ergot-free ration until the trial finished on Day 153.

Steer management, observation and sampling

Individual feed intakes were recorded and feed supplied was adjusted on a daily basis to slightly exceed individual appetite in order to achieve *ad libitum* intake. Residues were removed once weekly and weights recorded.

Steers were closely observed at least twice daily. Once each week, between 0800 and 1000 hours, they were weighed using calibrated electronic scales. Rectal temperatures of the steers were recorded at regular intervals, during the weighing process, and once on Day 17 at 1400 hours. On a single occasion between 1430 and 1500 hours on Day 35 (3 March) when ambient temperature was 32°C and temperature–humidity index (THI) was 76, respiration rates and any incidences of excessive salivation (drooling) were recorded for all steers. At 0900 hours on Day 70 (7 April), rectal temperatures were recorded with a digital thermometer (Beiersdorf Australia Ltd, North Ryde, NSW, Australia) and skin temperatures were recorded at three locations with a Light Touch Infrared Thermometer (Model LTX-1 Standard, Exergen Corporation, Watertown, MA, USA) at the inner surface of the ear ~50 mm from the end of the pinna, at the surface of the last rib and at the lateral surface of the coronet of the left hind limb.

Prior to ergot feeding and at least at 4-week intervals (more frequently on some occasions), steers were bled from the jugular vein and blood was collected into separate tubes containing EDTA or lithium–heparin. Samples were kept on ice until whole blood (in EDTA) was submitted for haematological examination. Plasma was removed from the lithium–heparin-preserved blood by centrifugation within 4 h and frozen at −20°C until prolactin concentrations were assayed.

Environmental conditions

Climatic data were accessed from the local Bureau of Meteorology recording station. The THI was calculated on an hourly basis throughout the experiment, using the following

formula: THI = ta + 0.36dp + 41.2, where ta is dry-bulb temperature and dp is dew-point temperature. Daily and weekly averages were then calculated from the hourly data.

Source and alkaloid content of ergot

Several tonnes of ergot-rich sorghum grain was obtained from a dairy farm in central Queensland, where severe reduction in milk production had occurred (Blaney *et al*. 2000*c*, dairy herd 1). This grain was regularly sampled while it was being augered from the delivery truck into a storage silo, and these samples were well mixed and combined to form an analytical sample of 2 kg. Ergot content of this grain was estimated by visual separation of 200 g subsamples to contain ~60 g/kg of ergot bodies, although estimates varied (30–80 g/kg) between samples and analysts. Sorghum ergot is commonly present in grain as immature ergot bodies (mixed sphacelial and sclerotial tissues), and the correlation between ergot and alkaloid contents is poor (Kopinski *et al*. 2008) because alkaloid production is confined to sclerotial tissue. The remaining sample was milled and the total alkaloid content of the ergot-rich and ergot-free sorghum (see below) was determined by a combination of HPLC and spectroscopic methods as described by Blaney *et al*. (2003). Estimates of total alkaloid concentration determined by spectrometry approximately matched the sum of individual alkaloid concentrations estimated by HPLC, within analytical variation. The average alkaloid concentration in grain (mg/kg, as fed basis) was: DHES, 21; DHEC, 2.5; FC, 1.5; and total, 25. Traces of pyroclavine (≤0.5), the optical isomer of FC, might also have been present. No other ergot alkaloids were detected (<0.1 mg/kg), including ergotamine and others produced by Australian rye ergot (Blaney *et al*. 2009). A source of sorghum that was not ergot-infected (ergot-free sorghum) was also acquired for mixing with the ergot-rich sorghum to provide treatment concentrations of alkaloid. Alkaloids were not detected in this ergot-free sorghum (<0.1 mg/kg).

Formulation of rations

The feedlot rations were based on a combination (90 : 10, w : w, as fed) of grain concentrate and roughage which was chaffed Rhodes grass (*Chloris gayana*) hay. The concentrate component of the ration included the following (g/kg, as fed): dry-rolled sorghum grain, 866; molasses, 56; cottonseed meal, 30; bentonite, 20; limestone, 12; urea, 10; ammonium sulfate, 2; and a mineral–vitamin–Rumensin© pre-mix, 4 (Elanco, Eli Lilly Australia, West Ryde, NSW, Australia). By calculation, the total alkaloid concentration in the grain concentrate based entirely on ergot-rich sorghum was ~19.5 mg/kg, and in the total ration 17.6 mg/kg.

The composition of the final ration components is given in Table 1. There were only small differences in composition between the various final rations, with a trend for starch content to decrease with increasing inclusion of ergot. The estimated metabolisable energy (ME) content was similar for all rations.

Nutritional analyses

Proximate analyses were conducted on the ergot-rich and ergot-free sorghum grain and the roughage. Dry matter (DM) content was determined by heating to a constant weight at 105°C under an atmosphere of nitrogen, using an automated LECO Thermogravimetric TGA 601 Analyser (LECO Corporation, St Joseph, Michigan, USA). The ash content was determined by further heating the dry samples in the Thermogravimetric Analyser at 600°C to a constant weight, and organic matter (OM) was determined by difference. Starch was analysed by conversion to glucose using a 2-step enzyme treatment, and colorimetric determination of the glucose with a glucose oxidase/peroxidase reagent. All enzymes and reagents were supplied in kit form from Megazyme available from Deltagen Australia. The enzymatic breakdown of the starch using a heat-stable α-amylase and amyloglucosidase is based on the procedures of McCleary *et al*. (1997). Crude fibre content was determined by the method of Moir (1971), using the Filtrex extraction tubes (Faichney and White 1983). Heating for both acid and alkali extractions was by boiling water bath for 30 min. Acid detergent fibre (ADF) concentration was determined using the method of Goering and Van Soest (1970), using the Filtrex apparatus. Neutral detergent fibre (NDF) concentration was determined using the method of Van Soest and Wine (1967) modified to use the Filtrex apparatus. Crude fat (ether extract) content was determined by Soxhlet extraction using hexane for

Table 1. Nutrient concentrations (dry-matter basis) in ration components and formulated grain concentrates
Metabolisable energy (ME) values were estimated by using formulae given in the text. ADF, acid detergent fibre; NDF, neutral detergent fibre; OM, organic matter; –, not determined

Parameter	OM (g/kg)	Starch (g/kg)	Crude fibre (g/kg)	NDF (g/kg)	ADF (g/kg)	Crude protein (g/kg)	Ether extract (g/kg)	ME (MJ/kg)
Ration component								
Rhodes grass hay	903	–	–	722	413	49	20	5.6
Ergot-free sorghum	989	690	27	80	–	89	34	14.0
Ergot-rich sorghum	975	555	45	121	–	122	33	13.6
Ergot (sphacelia/sclerotia)	907	277	115	273	–	134	62	12.4
Grain concentrates in rations								
Ration E0	955	631	27	–	–	137	31	13.4
Ration E1.1	960	637	27	–	–	130	34	13.5
Ration E2.2	954	605	33	–	–	142	34	13.3
Ration E4.4	949	610	30	–	–	138	32	13.3

16 h (Kent-Jones and Amos 1957). The samples were analysed for total nitrogen content by a combustion method (Sweeney 1989), using an ELEMENTAR RapidN analyser, calibrated using AR grade aspartic acid. Crude protein was estimated from total nitrogen, using the factor 6.25.

Metabolisable energy (ME) density was calculated in Rhodes grass hay (*in vitro* organic matter digestibility of 41.3%) using eqn 58 of MAFF (1975): ME (MJ/kg DM) = 0.15 digestible organic dry matter expressed as % of dry matter. *In vitro* digestibility was determined using a two-stage technique (Tilley and Terry 1963) as modified by Minson and McLeod (1972). For grain and concentrates, ME density was calculated using eqn 75 of MAFF (1975): ME = 0.12%crude protein + 0.31% ether extract + 0.05%crude fibre + 0.14%nitrogen-free extract, where nitrogen-free extract = 100 – (%crude protein + %ether extract + %crude fibre + %ash).

Blood analyses

Blood samples were assayed for haemoglobin, packed cell volume, erythrocyte count, mean corpuscular haemoglobin, mean corpuscular volume and leukocyte count using standard methods. Plasma prolactin concentrations were determined by radioimmunoassay (Downing *et al*. 1995) – the detection limit was 0.9 ug/L and results below this were given a value of 0.45 ug/L for statistical analysis.

Biometrical analyses

Analysis of variance (ANOVA) was used to compare the effects of treatments on rectal temperatures, feed intakes, growth and feed conversion, with block and treatment as factors in the model. A dummy covariate for small *versus* large pens was tried and found to be non-significant. The prolactin data were transformed to the ln(1+ concentration) scale before ANOVA, so as to stabilise the variance. Means were compared via the protected least-squares difference (l.s.d.) procedure operating at the 5% level of significance.

Results

By noon on the second day after offering rations containing 0, 4.4, 8.8 and 17.6 mg/kg alkaloids, there was almost complete feed refusal and signs of heat stress in ergot-fed groups. While all control steers consumed their ration, only three steers of eight offered 4.4 mg/kg, and none offered rations with higher alkaloid concentrations consumed appreciable amounts of feed, and these animals were selectively consuming roughage rather than grain. One steer became severely stressed to near collapse (rectal temperature 42.1°C) and was removed from the experiment (this steer appeared normal 2 days later).

After recovery on ergot-free rations and gradual re-introduction of lower alkaloid concentrations, there was no sudden feed rejection but there remained a clear association between high ambient temperatures and signs of heat stress in ergot-fed steers. The signs abated in the early morning, but reappeared with mild exercise (walking 50–100 m for weighing). This hyperthermia was displayed by high rectal temperatures, increased respiration rates, panting, drooling, mouth breathing, and in a few cases, standing in water troughs. Urination also appeared excessive.

Rectal temperatures at 0800–1000 hours are shown in Fig. 1 and are evaluated against the weekly average THI, and the number of hours each week when the THI exceeded 70. Hourly THI measured over Week 11, when the average weekly THI was 71, showed the daily minimum ranging from 62 to 69 at 0600 hours and the daily maximum ranging from 73 to 79 at 1300–1500 hours. The following week (Week 12), when average weekly THI was 68, showed the daily minimum ranging from 61 to 66 and the daily maximum ranging from 67 to 72.

As ergot was being gradually reintroduced to rations during Days 20–32, the number of hours/week where the THI exceeded 70 decreased from ~120 during Days 21–28, down to ~60 during Days 28–35. The rate then increased to ~95 h/week up to Day 63, and then declined again.

Rectal temperatures were significantly ($P < 0.05$) elevated in all groups receiving ergot over the E0 (control) group on Days 63 and 70 (Fig. 1) when the average weekly THI was ~70. However, 4 weeks later, when the average weekly THI had reduced to 63 and the number of hours exceeding 70 had reduced to <20 h/week, rectal temperatures of steers receiving ergot were similar to those of control steers.

Signs of hyperthermia were most severe at early afternoon, which corresponds with normal increases in body temperature during this part of the day when THI was at its maximum. While average rectal temperatures were 39.4°C at 0900 hours on Day 14, before ergot feeding, the average measured at 1400 hours on Day 17 before reintroduction of ergot averaged 41.1°C (there were no significant differences between treatment groups on either of these days).

At 1430–1500 hours on Day 35 (3 March), respiration rates for all groups ranged from 80 to 130 breaths/min, and the average for the control group was 100 compared with 111 for ergot-fed steers. Only one of the eight control steers was drooling and this steer had a respiration rate of 130 breaths/min. Only 2 of the 24 ergot-fed steers were not drooling, and both had respiration rates of 80 breaths/min.

Skin surface temperatures measured at 0900 hours on Day 70 (7 April) were related to rectal temperatures as follows: elevated rectal temperatures (40.7°C in ergot-fed steers *v.* 39.8°C in control steers) were paralleled by increases in temperature of the inner surface of the ear (36.5°C *v.* 35.0°C), but not by temperatures on either the rib surface (31.7°C *v.* 31.9°C) or hind feet (30.4°C *v.* 30.7°C), respectively.

Feed intakes (DM basis), growth and feed conversion rates are summarised in Table 2. It is clearer to interpret the data when divided into three periods: (I) the first 77 days when the average daily THI was above 70, and there were >40 h/week when the THI exceeded 70; (II) the next 42 days until the E0 steers were sent to slaughter and when the average daily THI was below 70, and there were <20 h/week when the THI exceeded 70; and (III) the final 35 days when steers previously fed ergot were given ergot-free rations. In both Periods I and II, feed intakes for ergot-fed groups were significantly reduced relative to the E0 group (see Fig. 2). The depression in feed intake (liveweight basis) was greater for the E2.2 (22%) and E4.4 (22%) groups compared with the E1.1 group (12%) in Period I. In Period II, feed intakes increased across all treatment groups, but ergot treatments continued to significantly depress intakes by 18, 20 and 19%, respectively,

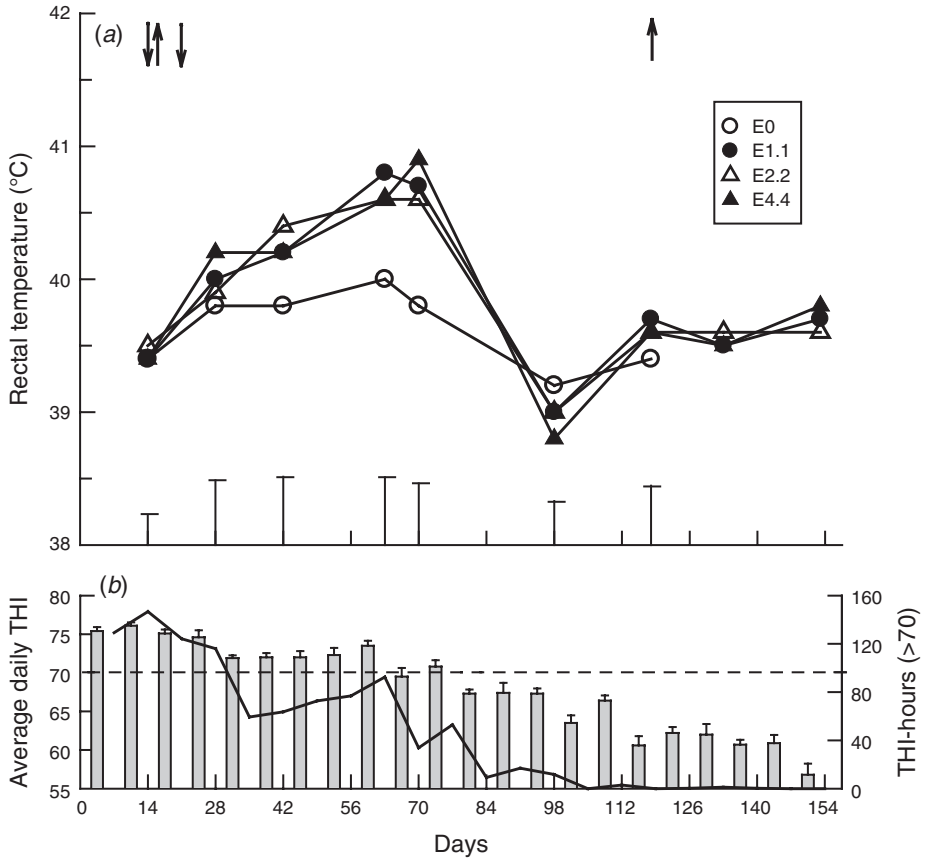

Fig. 1. (*a*) Changes in rectal temperatures of Hereford steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). Vertical bars represent the least significant differences (l.s.d., $P = 0.05$) for treatment means. The arrows indicate when ergot was included (↓) or withdrawn (↑) from the diet. (*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 70.

compared with control (E0) steers. There were no significant differences in intake between the three ergot-treated groups in Period III, after ergot had been removed, although there was an indication ($P > 0.05$) that the E4.4 group showed greater recovery in feed intake than the other groups (Fig. 2).

The liveweight trends are illustrated in Fig. 2. The cumulative liveweight change pattern was relatively linear over the total period for the E0 group (average gain 1.45 kg/day) but with steers receiving ergot there appeared to be a point of inflection in the liveweight change line at Day 77, when the THI dropped below 70, and growth rates increased. These observations are summarised in Table 2. Liveweight changes tended to follow the same trends as feed intakes, although growth rate ranked in the inverse order to alkaloid concentration in the rations. In Period I, growth rates were markedly reduced with ergot addition to the diets, by 0.47 (E1.1), 0.65 (E2.2) and 0.89 (E4.4) kg/day, compared with E0 steers. Growth rates in Period II were much higher in ergot-treated steers compared with Period I but the E2.2 and E4.4 groups still had lower gains ($P < 0.05$) than the E0 group, with no difference in growth rate between the E1.1 and E0 groups. Over both periods, the trends were similar to those described for Period I alone. The feed conversion ratios over Periods I and II combined were highly variable, especially for groups receiving ergot, and while there was a trend for worsening conversion efficiency in ergot-fed groups, the differences between treatments were not significant.

The plasma prolactin results are shown in Fig. 3. Prolactin concentrations gradually rose from ~70 to ~150 ug/L in the E0 group over the course of the experiment. Inclusion of ergot in the diet was associated with a significant ($P < 0.05$) depression in plasma prolactin concentration compared with the E0 treatment at all sampling times. The depression was greater in the E2.2 and E4.4 groups than in the E1.1 group, and concentrations in the E2.2 and E4.4 groups were mostly below the limit of detection (<0.9 ug/L) from Day 42 to Day 118. There was a large increase in prolactin concentrations in the ergot-treated groups at the sampling after ergot was removed from the rations.

Haematological parameters were within the normal range overall, but there were trends ($P > 0.05$) for increases in haemoglobin, packed cell volume, erythrocyte count and leukocyte count, and decreases in mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular

Table 2. Effects of varying concentrations of sorghum-ergot alkaloids on liveweight, feed dry matter (DM) intake, liveweight gain and feed-conversion ratio of steers

After a 24-h exposure to higher alkaloid levels on Day 14, ergot was withdrawn and then gradually increased by Day 35 to the alkaloid concentrations shown. Within rows, means followed by the same letter are not significantly different. n.s., not significant

Variable	Ergot alkaloids in ration (mg/kg)				l.s.d.
	0	1.1	2.2	4.4	($P = 0.05$)
	Period I (Days 14–77)				
Day 14 liveweight (kg)	306.0	302.5	305.6	306.0	n.s.
Feed DM intake (kg/day)	9.74a	8.13b	7.13bc	7.00c	1.02
Feed DM intake (% of liveweight/day)	2.81a	2.47b	2.18c	2.20c	0.27
Liveweight gain (kg/day)	1.30a	0.83b	0.65bc	0.41c	0.30
Day 77 liveweight (kg)	388.2a	354.9b	346.8bc	332.1c	17.2
	Period II (Days 77–118)				
Feed DM intake (kg/day)	11.51a	8.64b	8.20b	7.98b	1.16
Feed DM intake (% of liveweight/day)	2.72a	2.24b	2.18b	2.21b	0.21
Liveweight gain (kg/day)	1.67a	1.51ab	1.38b	1.33b	0.25
Day 118 liveweight (kg)	456.7a	416.7b	403.4bc	386.5c	21.1
	Periods I and II (Days 14–118)				
Feed DM intake (kg/day)	10.43a	8.34b	7.55b	7.39b	1.01
Feed DM intake (% of liveweight/day)	2.74a	2.31b	2.13b	2.13b	0.24
Liveweight gain (kg/day)	1.45a	1.10b	0.94bc	0.77c	0.21
Feed-conversion ratio (kg/kg)	7.2	7.6	8.9	10.6	n.s.
	Period III (Days 118–153)				
Feed intake (kg/day)	–	9.95	9.42	10.26	1.34
Feed DM intake (% of liveweight/day)	–	2.33	2.24	2.52	0.27
Liveweight gain (kg/day)		1.39	1.52	1.72	n.s.
Day 153 liveweight (kg)	–	465.4	456.4	446.9	n.s.

haemoglobin concentration in ergot-exposed groups (results not shown). Overall, these changes were consistent with mild dehydration as a result of hyperthermia.

Discussion

Effect of sorghum ergot on thermoregulation

The effects of sorghum ergot were clearly related to hyperthermia, with increased rectal temperature, excessive salivation, mouth breathing and panting, increased respiration rates, standing in water troughs and excessive urination. Signs could be exacerbated by mild exercise, such as walking 50–100 m for weighing in the morning, but were worst at the hottest part of the day in the early afternoon. One steer at the point of collapse was withdrawn from the trial with a temperature of 42.1°C; a rectal temperature of 43°C is lethal. We measured average rectal temperatures of 41°C at 1400 hours, in the absence of ergot, but rectal temperatures of ergot-fed steers were ~41°C even in the early morning when the THI was only 68–70. The THI was originally developed by Thom (1959) to characterise heat stress for humans, but is now widely used to alert livestock producers to conditions that threaten animal well being. In the USA, three THI categories are used as an environmental management tool: alert (THI from 75–78); danger (THI from 79–83); and emergency (THI >84), when large mortalities can occur (Brown Brandl *et al*. 2005).

The conditions that limit animal production are more complex, and must take into account the number of hours each day that the THI exceeds any specific base value such as 75 or 79, and also the amount of overnight cooling that can allow animals to compensate. In Australia, various studies have used this THI formula to address the relationship between THI and production; e.g. milk production of dairy herds declined when the maximum daily THI exceeded 72 if shade was not provided, but if both shade and sprinklers were provided, a THI of 78 could be tolerated before milk production declined (Mayer *et al*. 1999). Investigations have also been reported into the effect of climate change on potential heat stress (number of days when the maximum THI exceeded 80) for beef cattle in southern Queensland (Howden and Turnpenny 1997). We observed hyperthermia in ergot-fed steers during periods when the average weekly THI was $\geq$70, and the hourly THI was $\geq$70 for >60 h/week, while control steers were only mildly affected. Since our experiments were conducted, more detailed models have been developed to more accurately measure heat load in feedlot cattle, incorporating solar load (through 'black body' heat absorption) and wind speed (Mader *et al*. 2006; Gaughan *et al*. 2008).

The hyperthermia syndrome observed here appeared very similar to that described in cattle consuming rye ergot (Peet *et al*. 1991; Schneider *et al*. 1996) and tall fescue (Oliver 2005). We also observed the other signs associated with ingestion of rye ergot and fescue alkaloids, *viz*. reduced feed intake, reduced growth rates, poor feed conversion and rough hair coat. Not all individuals were susceptible in our study as one control steer showed signs of heat stress while one steer fed the

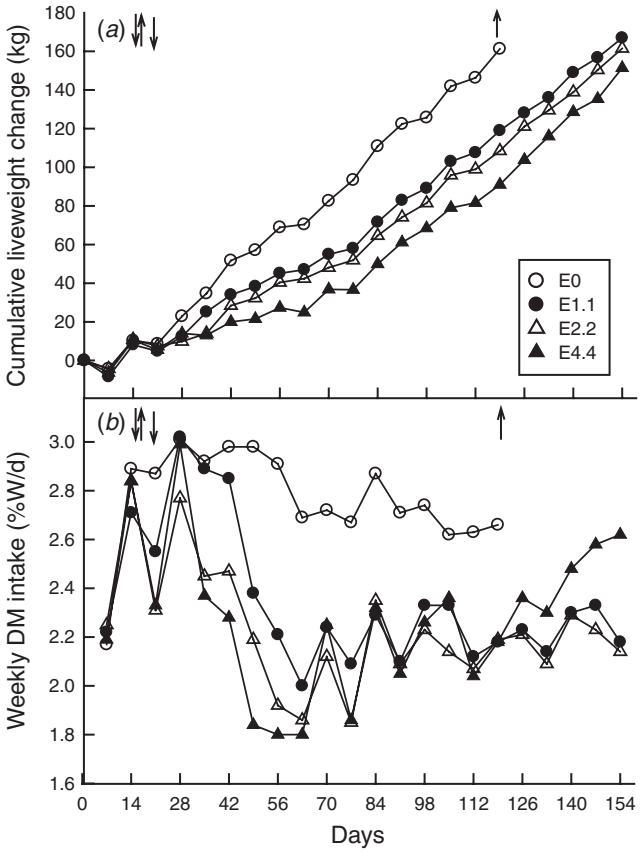

Fig. 2. Changes in (*a*) liveweight and (*b*) weekly feed dry matter (DM) intakes by steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). The arrows indicate when ergot was included (↓) or withdrawn (↑) from the ration.

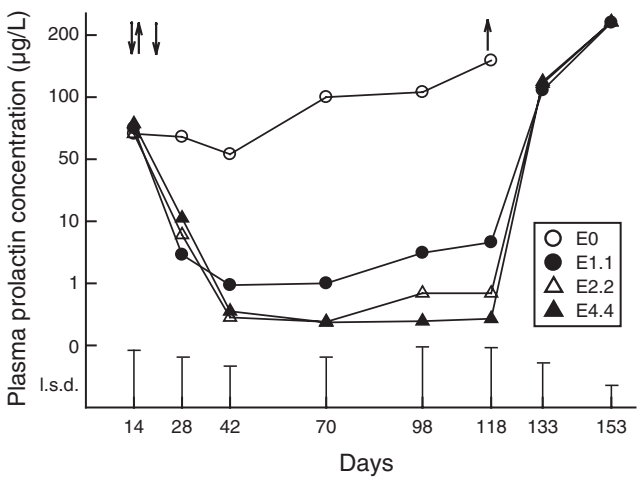

Fig. 3. Changes in the concentration of prolactin in the blood plasma of steers given sorghum-based feedlot rations containing varying concentrations of ergot alkaloid (see text for details of treatments). Concentrations plotted are treatment means (geometric means) back-transformed after the analysis of variance of ln(1 + concentration). The least significance difference (l.s.d., $P = 0.05$) bars are shown for each sampling time. The arrows indicate when ergot was included (↓) or withdrawn (↑) from the diet.

highest ergot level did not show signs. Cattle also show large individual differences in susceptibility to the alkaloids in infected tall fescue (Spiers *et al*. 1997).

We did not observe the highly lethal form of hyperthermia associated with rye ergot, as reported by Peet *et al*. (1991) and Bourke (2003) in feedlots in Western Australia and New South Wales, respectively, and which Bourke (2003) has associated with sun exposure. Ergotamine is the dominant alkaloid in Australian rye-ergot sclerotia (Blaney *et al*. 2009), and might have more severe impact on temperature regulation than DHES and other dihydro-alkaloids (as discussed below). However, in the present trial, apart from the initial high but short exposure, sorghum ergot was gradually introduced into rations and shade was provided, so no clear comparison can be drawn with the hyperacute rye-ergot intoxications in Australia. Bourke (2003) has suggested that other toxins from rye ergot (ergochromes, Franck 1980) might be involved, but it is not known if sorghum ergot produces ergochromes.

Effect of sorghum ergot on feed intake and growth

The growth rate of steers receiving the ergot-free, sorghum-based feedlot ration (1.45 kg/day) was consistent with those recorded previously with similar cattle and sorghum-based rations at the same research centre (J. A. Connell, unpubl. data). Based on calculations using the Australian feeding standards (CSIRO 2007), this growth rate would be achieved at the recorded feed intake if the ME of the ration was ~11 MJ/kg DM, instead of the 13.4 MJ/kg DM estimated from proximate analysis of the ration (Table 1). In fact, the value of 11 MJ/kg DM is more consistent with actual reported values for non-steam flaked sorghum grain (e.g. NRC 1996). Using this energy density across all treatments in the CSIRO (2007) calculations, the estimated growth rates for Periods I and II combined were: E1.1, 1.07 kg/day; E2.2, 0.90 kg/day; and E4.4, 0.90 kg/day. Thus, only in the case of the E4.4 treatment was there a discrepancy between estimated and observed growth rates (Table 2); this could be explained if the energy density of the E4.4 ration had been reduced to 10.5 MJ/kg DM, in consequence of dilution of the energy-rich components (starch) of the sorghum through ergot infection (Table 1). Consequently, most of the reduction in growth rate that we observed can be attributed simply to reduced feed intake. However, there was also a trend for worsening of feed conversion efficiency especially at the highest ergot inclusion rate, although differences between groups did not reach the point of significance.

Growth was depressed in all ergot-fed groups compared with the control group, consistent with the reduced feed and energy intakes, with the result that ergot-fed groups took at least 5 weeks longer than controls to reach a suitable slaughter weight of 455 kg. The lowest concentration at which such a growth depression occurs was not determined in the present study. The depression in growth rates of steers receiving 1.1 mg alkaloids/kg of feed (~2.5 g sorghum ergot/kg) relative to the control group was ~24% over the experimental period. There are little data on effects of rye ergot on lot-fed cattle for comparison. Dinnusson *et al*. (1971) recorded an 11% growth depression in beef steers fed 5 g rye ergot/kg of feed and some growth depression in steers receiving 1.5 g rye ergot/kg, but did not record alkaloid contents.

Effect of sorghum-ergot alkaloids on prolactin and other biogenic amines

While all of the mechanisms of ergot-alkaloid intoxication have not been clarified, it is widely accepted that most of the effects are due to interference with biogenic amine receptors through structural similarities to dopamine, serotonin and noradrenalin (see Oliver 1997; for a review).

The chief function of dopamine as a neurohormone is to inhibit release of prolactin by lactotrope cells in the pituitary, and binding of ergot alkaloids to dopamine-2 receptors (Larson *et al*. 1995) exacerbates this effect, so that reduced plasma prolactin concentration is a very sensitive indicator of ergot-alkaloid ingestion. Reaction of dihydro-alkaloids with receptors has been less studied, but Soskic *et al*. (1986) found that either hydrogenation of the 9,10 position of ergosine to form DHES, or epimerisation at C_8 did not affect dopinergic activity, although both in combination did decrease affinity for D_2 and D_3 receptors compared with DHES and ergosine, pointing to receptor stereo-specificity. This is consistent with our results, which showed severe prolactin depression in all ergot-fed groups compared with controls, and a 'rebound' effect after ergot was removed from the rations at Day 118. However, the increase in prolactin in control steers during the feeding period, while ambient temperatures and daylength actually reduced, is in contrast to other reports, and we can offer no explanation. Prolactin is known to fluctuate over 24 h, but steers were bled at the same time each day. Schams and Reinhardt (1974) showed there was a significant positive correlation between prolactin and number of daylight hours and ambient temperature (seasonal effects) in growing cattle. Fletcher *et al*. (1997) showed that prolactin concentration increased with ambient temperature in normal sheep, but not in grazing sheep ingesting 1 mg/kg ergovaline from infected ryegrass, and suggested that prolactin has a role in temperature regulation.

Also of relevance to the hyperthermia syndrome are the vasopressor effects resulting from interference with serotonergic and adrenergic receptors. Stimulation of adrenergic receptors affects smooth muscles including blood vessels and the resultant vasoconstriction is greater in peripheral veins than in arteries (Clarke *et al*. 1978), reducing peripheral blood flow and heat loss from skin. Klotz *et al*. (2007) showed that ergovaline and ergotamine had similar vasoconstrictor activity in the isolated bovine saphenous vein. Activation of serotonin-2 receptors also can have vasoconstrictor effects, and serotonin also has direct effects on hypothalamic thermoregulation and satiety centres, resulting in increased core body temperatures and appetite suppression (Oliver 2005). There is little specific information available about the interaction of dihydro-alkaloids like DHES with adrenergic and tryptaminergic (serotonin) receptors. However, both ergotamine and dihydroergotamine are partial agonists and antagonists to tryptaminergic receptors in some smooth muscles, and partial agonists and antagonists to α-adrenergic receptors in blood vessels and various smooth muscles in human subjects (Sanders-Bush and Mayer 2006). Consequently, it is likely that the dihydro-alkaloids of sorghum ergot have some vasopressor activity, but how this compares with the activity of ergotamine and ergovaline is unclear. Our finding of elevated rectal and inner-ear temperatures, but normal skin temperature at the rib and hind limb of steers fed sorghum ergot, is consistent with vasoconstriction.

Potency of sorghum-ergot alkaloids compared with those of rye ergot and tall fescue

Sorghum ergot produced effects that appeared very similar to rye ergot and tall fescue, and 1.1 mg sorghum-ergot alkaloids/kg of ration produced hyperthermia and had a severe impact on growth. The tolerance of cattle to rye-ergot alkaloids is not clear, but 1–2 mg/kg (mainly ergotamine and ergocryptine) in feed has been shown to produce hyperthermia (Bourke 2003; Blaney *et al*. 2009). By comparison, the threshold level of ergovaline in tall fescue known to produce clinical disease in cattle has been set at 0.4–0.75 mg/kg (Oliver 2005). This suggests comparable hyperthermic activity in steers of the alkaloids from all three sources.

The implication that DHES has similar activity to ergotamine and ergovaline is inconsistent with the known toxicity and pharmacology of 9,10-saturated alkaloids in man and other monogastric laboratory animals. For example, the LD_{50} (i.v.) in rabbits was 2.1 mg/kg for ergosine and 21 mg/kg for 9,10-dihydroergosine (DHES) (Griffiths *et al*. 1978). The dihydro-alkaloids have much less emetic effects than their parent, for example dihydroergotamine is used as a human pharmaceutical for migraine headache because it has much less emetic effect than ergotamine (and lower potency as a vasoconstrictor) (Sanders-Bush and Mayer 2006). One of Stoll's early works records that on saturating the 9–10 bond, action on smooth muscle, such as the uterus, is lost, while sympathicolytic action on organs under autonomic nervous system control is enhanced, and at the same time toxicity is considerably decreased (Stoll 1952). DHES has been patented as a human drug, with claims that it is an α-adrenergic blocker and serotonin antagonist. Other references show that DHES is a more potent dopamine antagonist than ergosine in some test systems (Pasic *et al*. 1987). This tends to suggest that DHES has less vasoconstrictor activity than ergosine, but more effect on dopinergic receptors. Ergo, it appears that DHES (once absorbed) would be more likely to affect lactation (Kopinski *et al*. 2008), and less likely to cause vasoconstriction, than alkaloids of rye ergot and tall fescue.

Neither lameness, nor gangrenous necrosis of extremities, was observed in any of our steers after 16 weeks. This syndrome has been reported with rye ergot and with tall fescue intoxication (fescue foot), but hyperthermia is far more common in warm conditions and some scientists have concluded that cold weather is necessary to produce gangrene (e.g. Ross *et al*. 1989). However, both syndromes can be expressed in some cases. In rye-ergot intoxication of cattle in South Africa (Schneider *et al*. 1996), initial hyperthermia was followed within ~30 days by lameness and signs of gangrenous necrosis of the feet, ears and tail. Fraser and Dorling (1983) reported cases of gangrenous ergotism from rye ergot in Western Australia, although hyperthermia is far more common in that region. The gangrenous syndromes may be a result of increased platelet aggregation caused by adrenergic receptor stimulation (Oliver 2005), leading to more severe restrictions on blood flow in

terminal limbs. These effects might vary with the alkaloid mixture ingested.

Browning and Leite-Browning (1997) showed that intravenous injection of ergotamine reduced the heart rate of steers, while ergonovine did not: both increased blood pressure compared with controls. In contrast, DHES has been patented as a human drug, with claims that it reduces blood pressure through increasing the strength of heart muscle contractions.

The effect of ergot alkaloids on ruminants is complex on several levels. Each ergot source contains a range of related ergopeptides and ergolines; ergovaline is the main alkaloid in tall fescue, but lower concentrations of ergine, ergosine, ergonine and their C_8 epimers are also present (Shelby 1997). These different alkaloids all have the same basic nucleus with similarities to the biogenic amines, and can potentially interfere with receptors. However, they can exert subtle differences in overall activity, probably due to stereochemical interaction and a different degree of binding with receptors (Seeman *et al*. 1985) as a result of the various constituents. Adding further complication is the partial bioconversion of ergopeptides such as ergovaline and ergotamine to simpler ergolines (lysergic acid, lysergol, ergonovine, ergine and others) in the rumen, such that any attempt to compare the toxicity of different sources must take into account the activity of the parent alkaloids and their bioconversion products on receptors.

Rumen digestion and absorption of ergot alkaloids

Hill (2005) provided a detailed review of the factors governing absorption of ergot alkaloids in ruminants, with reference to tall fescue alkaloids. Tall fescue contains mainly ergovaline, with smaller amounts of lysergic acid amide (ergine) and traces of clavines (assuming loline does not play a role in the syndrome we are discussing). Ergine is easily transported across the rumen wall, but ergovaline is not because it is not soluble in rumen fluid. However, a large proportion of the ergovaline might be biotransformed in the rumen to lysergic acid, ergonovine and other ergolines, which are then actively transported across the rumen wall (Hill *et al*. 2001). Residual ergovaline is then absorbed post-ruminally, where lipophilic compounds are more favoured for transport. After absorption, ergovaline is biotransformed in the liver to simpler compounds – ergolines. At any stage during livestock consumption of tall fescue, there is a mixture of ergovaline, ergine, ergonovine and other ergolines circulating in the blood, all or which can interact with biogenic amine receptors in highly complex ways.

In extrapolating these factors to sorghum ergot, it is pointed out that the dihydro-alkaloids are much more stable than their unsaturated counterparts, without undergoing spontaneous C_8 epimerisation as occurs with e.g. ergovaline to ergovalinine and ergotamine to ergotaminine (Hafner *et al*. 2008). They are also much more stable to light, because formation of the inactive lumi-derivatives of rye-ergot alkaloids through light-catalysed addition of water or alcohol also appears to be facilitated by the electron cloud around the unsaturated 9–10 bond in rye-ergot alkaloids (Bulej and Cvak 1999). Other differences in chemical characteristics include slightly greater water solubility of the dihydro-alkaloids.

Sorghum-ergot alkaloids are typically contained in soft structures composed of mixed sphacelial and sclerotial tissues (Blaney *et al*. 2003) rather than the denser, chiten-walled, mature sclerotia, and should present little barrier to ruminal digestion. Because DHES is more water soluble, it could be more easily absorbed through the rumen wall than ergovaline, yet also have sufficient lipid affinity to be absorbed post-rumenally, as with ergovaline. With regard to bioconversion (in the rumen or liver), the literature suggests that both 9–10 unsaturated and saturated ergopeptides are similarly attacked at the peptide moiety, so DHES could also be transformed to simpler compounds in the rumen. However, the comparative rates of bioconversion have not been measured, and the greater chemical stability of DHES appears likely to slow bioconversion. Assuming both residual DHES and its conversion products (plus FC and DHEC and any derivatives) are absorbed and undergo further conversion in the liver, a mixture of 9–10 saturated ergopeptides and simpler ergolines would be produced. Our results show that the interaction of sorghum ergot alkaloids with receptors is overtly similar to that produced by rye-ergot and tall fescue alkaloids, yet there could be subtle differences worthy of further investigation.

Conclusions

Sorghum-ergot alkaloids in concentrations of 1.1 mg/kg and over had severely detrimental effects on the growth and fattening of steers under feedlot conditions, particularly when the average THI was $\geq$70. This alkaloid concentration was equivalent to ~2.5 g/kg (0.25%) ergot in the ration. However, as the relationship between alkaloid and ergot bodies in sorghum is very poor (Kopinski *et al*. 2008), ergot needs to be restricted in stockfeed for ruminants to levels of 1 g/kg (0.1%) or less in hot and humid conditions.

The tolerance of steers at lower ergot-intake levels and at lower temperatures requires investigation. Sorghum is grown in summer and tends to be utilised in northern Australian feedlots in the autumn/winter months, when tolerances might be higher. More investigation is also warranted into the susceptibility of *Bos indicus*-cross cattle, which have greater heat tolerance and are widely raised in the northern regions of Australia.

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