EFFECT OF A 3-DAY FAST ON STRESS HORMONES IN SHEEP WITH LOW OR HIGH STAPLE STRENGTH

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SUMMARY
This study measured hormone concentrations to determine whether sheep with a history of producing sound wool have a greater resistance to the stress of a 3-day fast. Fasting resulted in increased plasma cortisol concentrations and decreased urinary excretion of norepinephrine and dopamine, while excretion of epinephrine was unchanged. The high SS sheep had higher urinary excretion of epinephrine before and during the fast, and higher plasma concentrations of cortisol when fed above maintenance after the fast, but both groups responded similarly to the fast. Although fasting affected stress hormones, this did not translate into effects on follicle shutdown or minimum fibre diameter.

Keywords: Wool, epinephrine, norepinephrine, cortisol, follicle shutdown

INTRODUCTION
Much of southern Australia has a Mediterranean climate with mild wet winters and dry hot summers. Annual pastures are a feature of this environment and are characterised by large seasonal fluctuations in feed quantity and quality leading to large seasonal amplitudes in liveweight, wool growth and reduced staple strength (SS) in sheep grazing these pastures (Schlink et al. 1999). SS is the second most important determinant of the value of raw wool, after fibre diameter.

Low SS has been considered to result from stress (Peter et al. 1993; Behrendt 1994), although more recent controlled studies have thrown doubt on this belief (Mata et al. 1999; Schlink et al. 1999). However, experimental infestation with blowfly larvae caused low SS, accompanied by increased plasma concentration of cortisol (Walkden-Brown et al. 2000). Schlink et al. (2002a) showed that cortisol injections for more than 3 days significantly reduced SS by increasing the rate of follicle shutdown without the decrease in fibre diameter usually associated with reduced SS. Follicle shutdown has been associated with low SS in grazing sheep (Schlink and Dollin 1995; Hynd et al. 1997). Hynd et al. (1997) found that the peak in follicle shutdown occurred approximately one month after the opening rainfall of the wet season in autumn, and became more severe with increasing stocking rates.

These results indicate that severe stress resulting in an increase in plasma cortisol concentrations may result in follicle shutdown and low SS. However, it is not clear whether milder stress can affect SS. The present study examined the response of the stress hormones cortisol and epinephrine to a 3-day period without feed, although water was available. The study was carried out in sheep with a history of low or high SS to detect whether they differed in responsiveness to this stress.

MATERIALS AND METHODS
Twenty-four wethers (12 low and 12 high SS), initial liveweight of 62.7 kg, were selected from flocks used to establish the genetic parameters of wool SS (Howe et al. 1991). The low and high SS groups averaged 18.4 and 34.0 N/ktex, respectively, based on two previous annual shearings at the Great Southern Agricultural Research Institute, Katanning, Western Australia. Animals were brought into an animal house, and fed individually as described elsewhere (Schlink et al. 2002b). In brief, during days 0 to 67, each staple strength group was divided into two sub-groups (n = 6) fed either to liveweight maintenance or 8% loss of starting liveweight over that period. Feed was withdrawn on days 68 to 70, but animals were given free access to water, after which all sheep were fed 1430 g DM/day for 11 weeks and then released to the field for four months. Mid-side wool samples were collected at shearing (Schlink et al. 2002b). The work was carried out with approval of the CSIRO Floreat Park Animal Ethics Committee.

Total 24 h urine collections were made on 5 occasions: once 6 days before the fast (day 61), on each day of the fast (days 68, 69 and 70), and again 9 days after re-feeding (day 79). Urine was collected
into plastic bottles containing 80 ml 6% acetic acid and 0.5 g sodium metabisulfite, and sub-samples were stored at -20°C until assayed. Urinary free epinephrine, norepinephrine and dopamine were separated on an ODS reverse phase column using high pressure liquid chromatography and quantified with electro-chemical detection, using the method described in “Urinary catecholamines by LCEC” provided by Bioanalytical Systems Inc (W Lafayette, IN 47906, USA).

Indwelling jugular catheters were inserted 24 h before the initiation of blood sampling. During the fast, blood samples were collected every hour for 10 h starting at 0800 h. Additional plasma samples were collected on day 61 and day 79, as described by Schlink et al. (2002b). Blood was collected into heparinised tubes, centrifuged and plasma stored at -20°C until assayed. Plasma cortisol concentrations were measured using the radio-immunoassay (Atkinson and Adams 1988).

Statistical analyses were carried out by univariate or repeated measures analysis of variance as appropriate. Values for epinephrine and dopamine were transformed to logarithms to more closely approximate the normal distribution. If there were no statistically significant interactions between treatment and time, the treatments were pooled within sampling to simplify comparisons of the two sampling dates, which were examined by repeated measures analysis of variance.

RESULTS
The effects of feeding treatments are shown in Fig. 1. Sheep that had been fed below maintenance excreted less of both norepinephrine and dopamine than animals fed at maintenance (day 61, \( P < 0.05 \)), but epinephrine excretion was unaffected. During fasting, reduced amounts of norepinephrine (\( P < 0.05 \)) and dopamine (\( P < 0.01 \)), but not epinephrine, were excreted in urine. Nine days after the animals were re-fed (day 79), only the excretion of dopamine increased significantly (\( P < 0.05 \)) in animals fed below maintenance previously.

![Figure 1. Effect of feeding level on urinary excretion of dopamine, epinephrine and norepinephrine in sheep feed below maintenance (diagonal) or at maintenance (cross-hatch) for 61 days, daily samples in the sheep fasted for 3 days (days 68, 69 and 70), and then 9 days after re-feeding (day 79). \( *P < 0.05 \), difference between feeding groups within each day.](image-url)
There were no interactions between feeding treatment and SS group, so the effect of the SS group is shown separately in Fig. 2. Repeated measures analysis indicated that the high SS group excreted more epinephrine in the urine overall (P < 0.05). Univariate analysis indicated that this difference occurred primarily before fasting (day 61) and during the first two days of the fast (Fig. 2). The excretion of norepinephrine and dopamine was similar in the two SS groups.

Feeding level before or after the fast did not affect concentrations of cortisol in plasma, but concentrations increased substantially during the fast (P<0.01; Fig. 2). Plasma concentrations of cortisol were greater in the high SS group after fasting (Fig. 2). There was no interaction between SS group and feeding treatment.

**Figure 2. Effect of staple strength group (low SS, diagonal bars; high SS, cross hatched bars) on urinary excretion of epinephrine and plasma concentration of cortisol, when fed at or below maintenance (days 61 and 64, respectively), fasted for 3 days (day 68, 69 and 70) and re-fed for 9 or 13 days respectively (days 79 or 83). * P < 0.05, ** P < 0.01 difference between SS groups within each day.**

There were no significant effects of SS group on fibre diameter at the point of break (14.4 and 15.2 μm for low and high SS groups respectively, P = 0.12), or prevalence of shed fibres at the point of break (2.8 and 3.6% for the low and high maintenance group respectively, P = 0.48). Sub-maintenance feeding reduced fibre diameter at the point of break (14.2 and 15.3 μm for sub-maintenance and maintenance fed groups respectively, P < 0.05).

**DISCUSSION**

The substantial increase in plasma cortisol concentration during the 3-day fast (Fig. 2) is consistent with a “stress” effect of fasting, but it is likely that the increase was not really due to stress. The other adrenal hormone associated with stress, epinephrine, did not increase. Furthermore, plasma concentrations of cortisol are strongly affected by the proportion of the circulation that flows through the liver (Panaretto 1974), and splanchnic blood flow would be reduced in unfed animals. Therefore, it is probable that the increased plasma concentration of cortisol resulted not from increased production by the adrenal gland, but from reduced clearance rate of cortisol from plasma by the liver. It is possible that the reduced excretion of norepinephrine and dopamine during the fast (Fig. 1) was also due to decreased clearance of catecholamines by the kidneys resulting from reduced splanchnic flow. Whatever the mechanism, there was no evidence that catecholamine activity was increased by fasting.
There was no hormonal indication that the low SS sheep were more sensitive to stress effects of fasting. Scobie et al. (1994) found that even small increases in epinephrine or norepinephrine produced a significant decline in mitotic activity in the wool follicle. Norepinephrine is produced mainly by the sympathetic nervous system, and affects metabolism primarily by promoting lipolysis (Rayner 2001). Dopamine is also a neurotransmitter, but its role in metabolism is unclear. Epinephrine is secreted by the adrenal gland, and increases the metabolic rate in sheep (Alexander and Bell 1982) and humans (Ratheiser et al. 1998). The higher excretion of epinephrine in the high SS sheep fed below maintenance or fasted indicates that they maintained a greater metabolic rate during this period, a finding which is consistent with the greater rate of protein synthesis observed in high SS sheep when fed below maintenance (Adams et al. 2000). Sheep with enhanced secretion of epinephrine by the adrenal gland during feed restriction may therefore have improved SS, although it is not clear why this apparently was insufficient to affect SS through decreasing the mitotic rate in the wool follicle.

Although plasma cortisol concentrations were increased substantially during the fast, there was little evidence of follicle shutdown and fibre shedding in this study. This is consistent with the finding that sheep needed to be treated with cortisol for longer than 3 days to cause significant follicle shutdown (Schlink et al. 2002a). Furthermore, the 3-day fast did not produce a significant change in fibre diameter in either nutritional or SS groups, despite large nutritionally induced differences in fibre diameter at the start of the 3-day fast (Schlink et al. 2002b). Similarly, Thwaites (1972) found that feeding for 14 days at 10% maintenance had no effect on mid-side patch fibre diameters. In field studies, Schlink et al. (2000) found no effect of the "break of season" on fibre diameter, and Mata et al. (1999) could not detect a specific weakness in staples at the break of season. We conclude that fasting events per se have little effect on wool growth rate and are unlikely to cause sufficiently prolonged elevations in plasma cortisol to affect SS. Despite the wide spread belief that low SS is due to "stress", this study indicated that short-term stresses have negligible effects on SS.

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REFERENCES


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