### SALIVARY CORTISOL AND BEHAVIOURAL INDICATORS OF STRESS IN SHEEP

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### SUMMARY

Salivary cortisol values provided a means of assessing the relative effects of acute stressors on sheep flocks over a range of husbandry situations, despite the fact that individual animals were not consistent in their responses. to different acute stressors. Various behavioural measures on individual animals were not closely related to salivary cortisol in this study. Using flock salivary cortisol or plasma free cortisol as a single indicator of stress gave the following ranking of acute stressors in descending order: surgical procedures (mulesing, castration, docking), rough transport, minor surgical treatments (pizzle dropping, teeth grinding), shearing, yarding with dogs, other yarding. Keywords: stress, sheep, handling, cortisol, behaviour .

#### INTRODUCTION

Previously we reported on the use of salivary cortisol measurement (Fell et al. 1985) as an indicator of stress due to management practices in sheep and calves (Fell and Shutt 1986). Since then we have been collecting data from a wide range of sheep husbandry situations and attempting to add other behavioural and physiological indicators to provide a broader view of the stress response.

Behavioural assessment of stress is obviously important, but its relationship with physiological stress is not clear (Blackshaw 1986). In this paper we compare salivary cortisol values and examine the relationship between salivary cortisol and some behavioural parameters of stress and the growth rate and wool production of individual animals.

# MATERIALS AND METHODS

Salivary sampling and cortisol assay (Fell et al. 1985) and plasma cortisol measurement (Shutt et al. 1987) were as described previously.

In the 'main study the experimental animals were a uniform flock of 63 Merino x Border Leicester lambs which were born within a 6-day period. They were studied at various times from birth to their first shearing at 11 months of age., All salivary sampling was done in the same race or small pen at our Unit at Richmond. 'Behavioural responses were also recorded to measure' vitality at birth i.e. time. to stand, time to suckle; field activity in an open field test at 3 weeks and 10 months of age; 'and the flight distance or reaction to a person approaching the lambs in a large yard. From this flock, 50 lambs were also subjected to various lamb marking procedures as described by Shutt et al. (1987). Observations were made of the time spent struggling while in the marking cradle, and while the operation was being performed, and. also the number of bleats during these times. The productivity measures on these animals were growth rate and greasy fleece weight. Heart rate and respiration rate of individual animals were recorded with the aid of a stethoscope for 30 seconds immediately after catching the animal.

Other studies involved the sampling of a few animals from various flocks, throughout N.S.W. In each case the sheep were held in a race for sampling.

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#### RESULTS

In the main study of 63 lambs, salivary cortisol and behavioural data from individual animals were found to be virtually unrelated. There were no significant correlations between individual salivary cortisol values and other parameters. None of the behavioural measures nor salivary cortisol was significantly correlated with growth rate or fleece weight. The mean ( $\pm$  S.D.) greasy fleece weight was 2.6  $\pm$  0.39 kg and the growth rate was 82  $\pm$  14 g/day. There was considerable variation in time to stand (20  $\pm$  11 min), time to suckle (31  $\pm$  18 min) and field activity; less in birth weight (4.3  $\pm$  0.35 kg). There were significant (p<0.05) positive correlations between time to stand and time to suckle (r=0.75), growth rate and fleece weight (r=0.50) and birth weight and fleece weight (r=0.39). There were no significant differences between females and males in salivary cortisol nor in behavioural parameters, although males had a 15% longer time to stand. Twins differed significantly (p<0.01) from singles in that twins had 19% lower birth weight, 1 1% lower fleece weight and 36% greater field activity at 3 weeks of age. There was an indication of some correspondence, but in 10 lambs only, between flight distance and heart rate (r=0.7 1).

Table 1. Salivary, cortisol. in an experimental flock of lambs exposed to various treatments between 8 weeks and-1 0 months of age

Treatment	'n	Salivary cortisol (nmol/l) Mean <u>+</u> S <b>.</b> D <b>.</b>	Percentage >10 nmol/l
4 h after weaning (at 8 weeks old)	59	2.5 <u>+</u> 0.9	0
Control (7 a.m.)	59	3.0 <u>+</u> 1.9	.1
Control (10 a.m.)	59	3.3 <u>+</u> 2.2	
Cold <sup>A</sup> (7 a.m. Day 2 post-shearing)	58	3.6 <u>+</u> 3.2	3
Control (first yarding)	58	4.4 <u>+</u> 2.8	5
15 min after jetting	10	4.9 <u>+</u> 3.6	10
Cold <sup>B</sup> (7 a.m. Day 1 post-shearing)	57	5.1 <u>+</u> 5.2	11
15 min after isolation in open field test	61	5.9 <u>+</u> 3.6	15
15 min after sham shearing	10	6.1 <u>+</u> 5.2	20
After 1 h steady highway transport	47	6.2 <u>+</u> 5.2	17
After 30 min stop-start transport	47	12.4 <u>+</u> 7.6	53
15 min after shearing	59	13.8 <u>+</u> 8.5	61

A Frost. Minimum temp. -1.0°C.

<sup>B</sup> Heavy Frost. Minimum temp. -2.5°C.

The flock salivary cortisol data are summarised in Table 1. A range of -handling treatments is presented in ascending order of mean salivary cortisol or percentage of the flock showing a stress response (i.e. >10 nmol/l). An examination of individual responses showed that it was generally not the same animals which were consistently showing responses >10 nmol/l to different stressors.

Shearing and stop-start. transport produced the largest response of these comparatively mild stressors. Sham shearing carried out later gave a much smaller response. During the sham shearing (n=10) it was also observed that there was a 20% drop in mean heart rate (from 123 to 98 beats/min) and 'a 41% drop in mean respiration rate (from 118 to 70/min) while the sheep were being held.

Treatment	Struggling in cradle	g time (sec) during op.	Total no. in cradle	of bleats during op.	Plasma free cortisol (nmol/l) 15 min after treatment
Controls	13 <u>+</u> 11	0	3	0	13 <u>+</u> 6
Docking	13 <u>+</u> 8	2 <u>+</u> 1	13	1	27 <u>+</u> 19
Docking + castration	14 <u>+</u> 9	4 <u>+</u> 3	14	3	40 <u>+</u> 13
Docking + mulesing	40 <u>+</u> 6	29 <u>+</u> 4	8	5	46 <u>+</u> 19
Docking + castration + mulesing	26 <u>+</u> 9	19 <u>+</u> 9	54	42	61 <u>+</u> 22

Table 2. Behavioural and hormonal responses (mean,  $\pm$  S.D.) of lambs exposed to various surgical procedures at 4 weeks of age

The struggling and bleating during lamb marking are compared with plasma free The highest struggling time corresponded with the second highest cortisol in Table 2. hormonal response while there was a very high vocalisation score for the group with the maximum cortisol.

Table 3 Salivary cortisol in sheep from different flocks in various unrelated situations throughout N.S.W.

Treatment	n	Salivary cortisol (nmol/ Mean <u>+</u> S.D.	'l) Percentage >10 nmol/l
Ewes 1-8 h after mating	15	4.9 <u>+</u> 0.6	. 0
Ewes 15 min after mating	2	5.5	0
Ewes 15 min after yarding <sup>A</sup>	6	7.3 <u>+</u> 3.4	17
Ewes 15 min after yarding with "wide" dog <sup>A</sup>	6	9.4 <u>+</u> 5.4	33
Ewes 15 min after individual confinement with dog <sup>B</sup>	45	9.7 <u>+</u> 5.2	40
Ewes 15 min after yarding with "close" dog <sup>A</sup>	6	10.5 <u>+</u> 4.7	50
Wethers 15 min after yarding with dogs, strong wind and dust	20 ·	13.9 <u>+</u> 9.2	<i>5</i> 0
Ewes 15 min after teeth grinding <sup>A</sup>	6	14.8 <u>+</u> 4.7	83
Wethers 15 min after pizzle dropping <sup>C</sup>	6	20.9 <u>+</u> 14.0	100
Ewes after 30 min rough transport <sup>D</sup>	8	21.9 <u>+</u> 9.1	100

A Results kindly provided by Mr. G.A. Miller, 35 Henty Street, Culcairn, N.S.W. using the methods of Fell et al. (1985). The "wide" dog worked at a greater distance, from the sheep than the "close" dog..

B With Dr. P. Holst (N.S.W. Dept. Agric., Cowra). C 'With Mr. R. Marchant (N.S.W. Dept. Agric., Goulburn).

D From Fell and Shutt (1986).

Table 3 summarises salivary cortisol data from a variety of other sources arranged in ascending order.

# DISCUSSION

It appears from these results that flock mean salivary cortisol values (or the percentage of the flock showing > 10 nmol/l salivary cortisol) can be useful indicators when information is required about the relative effects of different management stressors which cause an acute stress response.

The plasma free cortisol and salivary cortisol values are comparable so it is apparent from a combination of Tables 1, 2 and 3 that the main surgical procedures gave the highest hormonal response to stress, followed by rough transport, minor surgical treatments, shearing and yarding. Severe fly strike was associated with similar plasma cortisol values to the maximum reported here (Shutt et al. 1987, unpublished).

Generally, the same animals did not show consistent responses to different stressors and the individual cortisol values were not correlated with productivity measures of individual animals in this small group. Part of the explanation for this may be the fact that the stressors were acute rather than chronic.

The type of behavioural assessments made in these studies were not closely related to salivary cortisol. The separate nature of the behavioural and physiological systems in the stress response has been discussed elsewhere (Burchfield 1985). However, behavioural responses constitute an important component of stress and, in our current work, more subtle behavioural effects of stress are being quantified and incorporated into a model showing psychological, physiological and behavioural interactions.

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