All animals were fed at 0900 h daily and refusals of feed were recorded. Wethers were given an injection of 16 mg of testosterone propionate in arachis oil into the biceps *femoris* muscle at 0900 h on days -1, 5 and 10. A 10 ml sample of blood was taken by jugular venepuncture on days -1, 5, and 10 at 1300. h and plasma was stored at  $-20^{\circ}$ C. Plasma concentrations of testosterone were assayed by a modification of the method of Risbridger et al. (1981). Briefly, the samples were extracted with hexane and chloroform (2 :1) and the dried extract was resuspended in phosphosaline buffer (0.2M, pH 7.2). The assay mix contained testosterone antiserum diluted to 1:10,000 and [<sup>3</sup>H] testosterone at approximately 10,000 cpm. After incubation for 1 hour at 30°C, the free and bound testosterone were separated using dextran coated charcoal (1% charcoal, 0.1% dextran T-70). Cross-reaction against 5<sup>a</sup>-dihydrotestosterone was 100%. Sensitivity of the assay was 0.05 ug/l and the intra- and inter- assay coefficients of variation were 6.0% and 15.0% respectively,

## RESULTS

Rams fed 2M rations consumed 94% of the ration offered (or 1.9M) and gained an average of 11.8% and 11.5% of their initial live weight in the 5 and 10-day periods respectively (Table 1). Those fed M rations gained 0.03% of their initial live weight during the 5-day period after which live weights remained stable while rams fed 1/2M rations lost 4.6% and 5.9% of initial live weight in the 5 and 10-day periods respectively (Table 1). Mean concentrations of testosterone were not significantly different when comparisons were made between nutrition groups or day of treatment (Table 1). There was no apparent trend and individual values varied considerably.

Table	1	Mean (+ s.e.) liveweight changes from the start of the treatment	nt
		period, concentrations of plasma testosterone measured on days -1,	5
		and 10 in rams fed $1/2M$ , M or 2M rations and co-efficient of	of
		variation c.v. of the testosterone	le testosterone

Group	n	Day of treatment	Liveweight change (kg)	Testosterone (ug/l)	с.v. (%)
		-1		2.8 <u>+</u> 0.68	76.4
1/2M	10	5	-3.0	4.3 <u>+</u> 1.07	74.4
		10	-3.8	3.0 <u>+</u> 0.74	78.0
		-1		5.1 <u>+</u> 1.35	83.9
м	10	5	1.8	$2.9 \pm 0.62$	64.1
		10	1.6	$2.3 \pm 0.44$	60.0
		-1		2.6 <u>+</u> 0.60	72.7
2M	10	5	7.1	$3.6 \pm 0.74$	65.0
		10	7.0	3.3 <u>+</u> 0.75	72.1

Wethers fed 2M rations actually consumed 75% of the ration offered (or 1.5M) and gained 0.1% and 4.5% of their initial live weight in the 5 and 10-day periods respectively (Table 2). Those fed M rations lost 2.6% of their initial live weight in the first 5 days and then gained 2.7% of their initial live weight to day 10 while wethers fed 1/2M rations lost 5.4% and 3.6% of their initial live weight in the 5 and 10-day periods respectively (Table 2). Proc. Aust. Soc. Anim, Prod. Vol. 18

Table 2 Mean (<u>+</u> s.e.) liveweight changes from the start of the treatment period and concentrations of plasma testosterone measured on days -1, 5 and 10 in wethers fed 1/2M,M or 2M rations and the co-efficient of variation (C.v.) of the testosterone data

Group	n	Day of treatment	Liveweight change (kg)	Testosterone (ug/l)	с.v. (%)
		-1	-	$4.2^{ab}_{ab} \pm 0.24$	17.9
1/2M	10	-1 5	-3.0	$4.6^{ab} \pm 0.47$	32.5
		10	-2.0	$5.9^{a} \pm 0.44$	21.0
		-1	-	$3.8^{b} \pm 0.35$	29.3
м	10	5	-1.5	$5.2^{ab} \pm 0.46$	28.2
		10	1.5	$5.4^{a} \pm 0.42$	24.4
		-1	-	$4.1^{b}_{-b} \pm 0.31$	24.0
2M	10	5	0.6	$4.3^{ab} \pm 0.56$	41.7
		10	2.6	$5.3^{a} \pm 0.63$	33.6

Means with different superscripts differ at P<0.05

While no main effect of nutritional treatment on peripheral testosterone concentration was evident (Table 2), when data from nutrition groups were pooled, it was found that the mean concentration of testosterone at day 10 was significantly higher than at day 5  $(5.5\pm0.30 v 4.7\pm0.30, P<0.05)$ . The difference between testosterone concentration at day 10 and day -1 was even greater  $(5.5\pm0.30 v 4.0\pm0.18, P<0.001)$  while the difference between concentrations at day 5 and day -1 was not significant (P>0.05). The coefficients of variation of testosterone data for rams were consistently higher than those for wethers (mean c.v.; 71.8% v 28.1% for rams and wethers respectively).

## DISCUSSION

There was no evidence of a relationship between the level of nutrition and the concentration of circulating testosterone in either rams or wethers. This indicates that the metabolic clearance rate of testosterone is not influenced by changes in the level of feed intake. In contrast, changes in nutritional level affect the metabolic clearance rate of progesterone (Parr et al. 1987a) such that the concentration of peripheral progesterone is inversely related to the level of nutrition, Mixed function oxidase enzymes, found in the liver have a high affinity for testosterone (Kuntzman, Lawrence and Conney 1965), but the Michaelis constant for testosterone is 61.5% of that for progesterone, which indicates that the rate of metabolism of testosterone is less than for progesterone. About 60% of the circulating testosterone is extracted during one passage through the liver in man (Horton and Tait 1966) while about 92% of the progesterone is removed during one passage through the sheep liver (Bedford, Harrison and Heap 1974). While recognising the difference in species, it is apparent that a considerable amount of testosterone metabolism may occur in extra-hepatic tissues.

The mean concentrations of testosterone in the wethers treated with 16 mg of testosterone propionate were higher than the concentrations of endogenous testosterone in the rams. The wethers were blood sampled 4 hours after injection when concentrations should have reached a maximum (D'Occhio and Brooks 1982). The dose of testosterone propionate was calculated to-provide peripheral concentrations equivalent to concentrations of endogenous testosterone in Merino rams (approximately 4 ug/l) during September (Bremner et al. 1984). It appeared that there may have been a residual effect of the testosterone propionate treatment as mean concentrations of testosterone

increased at the second and third treatments. Complete profile data are not yet available for these treatments but peripheral testosterone concentrations had returned to pre-injection levels 22 hours after injection of 8 mg of testosterone propionate in Merino wethers (D'Occhio and Brooks 1982). The consistently higher co-efficients of variation for testosterone in the intact rams would be expected given the pulsatile nature of testosterone secretion in the intact ram (D'Occhio, Schanbacher and Kinder 1982) and the relatively smooth testosterone profile that would be expected after the single injection of testosterone in the wethers. Despite the difference in variation between rams and wethers and the possibility of a residual effect in the testosterone propionate treated wethers, there was no evidence that changes in nutrition affected the metabolic clearance rate of testosterone in either rams or wethers.

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## REFERENCES

BEDFORD, C.A., HARRISON, F.A. and HEAP, R.B. (1974). J. Endocr. 62: 277.

BREMNER, W.J., CUMMING, I.A., WINFIELD, C.G., DE KRETSER, D.M. and GALLOWAY, D.B. (1984). In "Reproduction in Sheep", p.16, editors D.R. Lindsay and D.T. Pearce. (Australian Academy of Science: Canberra).

CUMMING, I.A., MOLE, B.J., OBST, J., BLOCKEY, M.A.deB., WINFIELD, C.G. and GODING, J.R. (1971). J. Reprod. Fert. 24: 140. D'OCCHIO, M.J. and BROOKS, D.E. (1982). Horm. Behav. 16: 383.

D'OCCHIO, M.J., SCHANBACHER, B.D. and KINDER, J.E. (1982). Endocr. 110: 1547.

HORTON, R. and TAIT, J.F. (1966). J. Clin. Invest. 45: 301.

KUNTZMAN, R., LAWENCE, D. and CONNEY, A.H. (1965). Mol. Pharmacol. 1: 163.

PARR, R.A., CUMMING, I.A. and CLARKE, I.J. (1982). J. Agric. Sci,, Camb. 98: 39.

PARR, R.A., DAVIS, I.F., FAIRCLOUGH, R.J. and MILES, M.A. (1987a). J. Reprod. Fert. 80: 317.

PARR, R.A., DAVIS, I.F., MILES, M.A., SQUIRES, T.J. and SIMPSON, G.J. (1987b). Proc. Aust. Soc. Reprod. Biol. 19: 132,

RISBRIDGER, G.P., KERR, J.B., PEAKE, R.A. and DE KRETSER, D.M. (1981). Endocr. 109: 1234.