The 'female effect' in Australian cashmere goats: effect of season and quality of diet on the LH and testosterone response of bucks to oestrous does

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The effects of season, diet and exposure to oestrous females on LH and testosterone secretion were examined in mature cashmere bucks to determine whether there is a seasonal cycle of LH and testosterone secretion, and whether this can be modulated by long-term differential nutrition and exposure to oestrous females. Three-year-old bucks were individually housed under natural photoperiod at 29°S 153°E and fed diets of high (crude protein 17.6%, metabolizable energy 8.3 MJ kg⁻¹) or low (crude protein 6.9%, metabolizable energy 6.6 MJ kg⁻¹) quality for 16 months *ad libitum* (*n* = 6 per treatment). Blood samples were collected to determine pulsatile LH and testosterone secretion immediately before experimental feeding, one month later, and every second month thereafter. Samples were collected for an 8 h period on successive days with the bucks isolated on the first day and each exposed to a single oestrous doe for the duration of the second day. In the absence of oestrous females, bucks exhibited a circannual pattern of secretion for both hormones with pulse frequency and mean concentrations highest in late summer and autumn and lowest in late winter and spring. Testosterone pulse amplitude followed a similar pattern, but LH pulse amplitude was highest in spring and lowest in autumn, indicating a seasonal shift in the relationship between the two hormones. Exposure to oestrous does increased LH and testosterone secretion depending on both season and diet. Responses were evident during summer, autumn and early winter, with bucks on a high quality diet exhibiting an earlier and more prolonged period of responsiveness than did bucks on a low quality diet, peaking in February compared with June. The magnitude of the LH and testosterone response was also significantly greater in bucks on a high quality diet. Weight loss during autumn appeared to reduce responsiveness in both treatments. These results demonstrate that there is a seasonal cycle in LH and testosterone secretion in mature cashmere bucks, and that nutrition and oestrous females are powerful modulators of the secretion of these hormones in a seasonally dependent way.

Introduction

In seasonal breeds of goat and sheep the introduction of males during the non-breeding season may induce cyclic reproductive activity in females well before the initiation of spontaneous cycling (Schinckel, 1954; Shelton, 1960). This response, known as the 'male effect' is widely used to advance and synchronize breeding in these species and its underlying physiology has been extensively studied (in sheep, Martin *et al.*, 1986; Signoret, 1990; in goat, Chemineau, 1987). Subsequently it was

discovered that there is an analogous 'female effect' on males of these species, with exposure to oestrous females resulting in rapid increases in LH secretion in rams (Sanford *et al.*, 1974; Yarney and Sanford, 1983) and goat bucks (Howland *et al.*, 1985). Although in rams this response is not dependent upon mounting or ejaculation (Gonzalez *et al.*, 1988a, b; Borg *et al.*, 1992), it is markedly reduced in the absence of physical contact with females (Gonzalez *et al.*, 1988a). Non-oestrous ewes elicit a reduced response (Tilbrook *et al.*, 1983; Gonzalez *et al.*, 1991a). In both rams (Yarney and Sanford, 1983; Schanbacher *et al.*, 1987) and bucks (Howland *et al.*, 1985) the endocrine response is seasonally dependent, being maximal during the non-breeding season, although reduced responses are evident during the breeding season (Gonzalez *et al.*, 1988a).

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Australian cashmere goats are derived from feral goats and exhibit marked reproductive seasonality (Harrington, 1982; Restall, 1992). However, while photoperiod appears to impose an annual cycle of reproductive activity in the female, both social (Restall, 1992; Walkden-Brown *et al.*, 1993a) and nutritional (Harrington, 1982) stimuli can modulate this pattern sufficiently to enable breeding out of season. Little is known about the impact of these environmental factors on males, although we have recently demonstrated that both improved nutrition and prior exposure to oestrous females enhance the ability of bucks to induce ovulation in seasonally anovulatory does (Walkden-Brown *et al.*, 1993b). This finding suggests that environmental influences acting on the male may be important in determining the success of out-of-season breeding.

In the present study we examined the role of photoperiodic, nutritional and social influences on LH and testosterone secretion in mature cashmere bucks over a 16 month period to determine whether (i) there is a seasonal cycle in LH and testosterone secretion in cashmere bucks maintained under controlled conditions, and whether (ii) this cycle can be modulated by nutritional and social stimuli.

Materials and Methods

Location and animals

The experiment was carried out at the Wollongbar Agricultural Institute (28°48'S, 153°25'E) during 1988 and 1989. Hours of daylight (sunrise to sunset) vary from 10.3 h at the winter solstice to 14.0 h at the summer solstice, while mean maximum and minimum temperatures range from 26.4 and 18.5°C in January to 17.6 and 8.9°C in July. The experimental animals were drawn from an unselected line of domesticated feral goats and comprised 12 34-month-old bucks all of which had previously been used for breeding. The history and management of the goat population at Wollongbar is described by Restall and Pattie (1989).

Experimental schedule

Bucks were individually housed in 2 m × 2.5 m pens under natural photoperiod for 16 months between 1 July and 13 October of the following year. All bucks were offered an introductory diet of 1.2 kg day⁻¹ of pelleted lucerne (*Medicago sativa*) and coarsely hammermilled pangola grass (*Digitaria decumbens*) hay in equal parts for 10 days before being divided into two treatment groups stratified on live-weight and serving capacity in three pre-experimental serving capacity tests (21 June, 29 June and 7 July). One group (Low) was fed a low quality diet of hammermilled pangola grass hay (crude protein 6.9%, metabolizable energy 6.6 MJ kg⁻¹) *ad libitum*, while the other group (High) was fed a high quality diet of pelleted lucerne (crude protein 17.6%, metabolizable energy 8.3 MJ kg⁻¹) *ad libitum* for the remainder of the experiment. A detailed description of the diets used and the feeding procedure is given by Walkden-Brown *et al.* (1994).

Intensive blood sampling for the determination of pulsatile LH and testosterone secretion occurred before the experiment (7 July), one month after the start of the experiment (9 August)

and every second month thereafter (11 October, 6 December, 7 February, 25 April, 6 June, 1 August and 3 October). Heparinized blood samples (5 ml) were collected from indwelling jugular catheters at intervals of 20 min and centrifuged immediately for 5 min at 2000 g before the plasma was decanted and frozen until required for radioimmunoassay. Samples were collected for an 8 h period (08.00–16.00 h) on successive days. On the first day bucks were sampled under normal conditions in their pens, but on the second day an ovariectomized doe in induced oestrus was placed with each buck following the collection of the first sample. The doe stayed in the buck pen for the remainder of the sampling period. Oestrus was induced in ovariectomized does by injecting 20 mg progesterone (Sigma Chemical Co., St Louis, MO) in 2 ml of vegetable oil on three occasions at intervals of 48 h, followed 48 h later by an injection of 100 µg of oestradiol benzoate (Intervet, Sydney) in 1 ml of vegetable oil. Oestrus generally commenced within 24 h of the oestradiol injection. Apart from during the test period no intact females were permitted within 100 m of the building housing the bucks.

Radioimmunoassay

LH was assayed in duplicate using a heterologous double antibody radioimmunoassay, based on the method of Martensz *et al.* (1976). Rabbit anti-ovine (o) LH antiserum (WLH-73, 0.1 ml, diluted 1:10⁵ with 1:600 normal rabbit serum) was added to plasma samples (0.2 ml sample + 0.5 ml 0.01 mol phosphate-buffered saline l⁻¹, PBS) and standards and allowed to incubate overnight at 4°C. The antiserum was kindly provided by H. Radford of the CSIRO Division of Animal Production, Prospect, and exhibited crossreactivities of < 3% with o-FSH, o-prolactin and o-ACTH, < 10% with o-GH and < 20% with o-TSH (Radford *et al.*, 1987). Standards were prepared in LH-free goat plasma using a caprine LH preparation (Henniaiwati, 1993). Tracer (0.1 ml, 15 000 c.p.m.) was then added and the tubes vortexed and incubated for 2 days at 4°C. Tracer was prepared using o-LH (Papkoff G3-223, immunopotency = 2.24 × NIH-oLH-S1) labelled with ¹²⁵I, as described by Martensz *et al.* (1976). Separation of the bound fraction was then achieved by adding donkey anti-rabbit serum (0.1 ml diluted 1:30 in 0.01 mol PBS l⁻¹ + 0.1% BSA) followed by overnight incubation at 4°C, centrifugation (at 2000 g for 50 min), aspiration and measurement of radioactivity in an automatic gamma counter.

The caprine LH standards exhibited a parallel displacement curve with NIH-oLH-S20 but it was displaced to the right giving a ratio of immunopotency of 1:4.8. The limit of assay sensitivity (± SEM) defined as the concentration of LH corresponding to a count 2 SDs above that for the zero standard was 1.33 ± 0.14 ng ml⁻¹. The mean nonspecific binding was 1.18 ± 0.03% and the mean zero binding was 38.0 ± 1.44%. Each assay contained six replicates of quality controls containing 3.0, 12.0 and 36.0 ng caprine LH ml⁻¹. The mean intra-assay coefficients of variation for these were 12.3, 8.1 and 6.3%, while the mean interassay coefficients of variation were 21.9, 7.8 and 6.6%, respectively. The mean recoveries for these quality controls were (mean ± SEM) 3.3 ± 0.25, 12.7 ± 0.35 and 37.6 ± 0.87 ng ml⁻¹, respectively. Serial dilution of samples

with LH free caprine plasma produced displacement curves parallel to the standard curves, indicating accuracy over the range of the assay.

Testosterone was assayed in duplicate using a single antibody radioimmunoassay after extraction with hexane, an adaptation of the method of Garnier *et al.* (1978). Antiserum (0.1 ml, dilution 1:12 500) was added to extracted samples dissolved in 0.1 ml PBS containing 0.1% gelatine. The antiserum was kindly provided by M.S.F. Wong, CSIRO Division of Animal Production, and exhibited crossreactivity of <1% with oestrogens and progesterone, 1.3% with androstenediol, 30% with 4-androstene-3 β -diol and 31% with dihydrotestosterone (Brown *et al.*, 1989). Tracer (1,2,6,7-[3 H]testosterone, Amersham, Sydney) was added 30 min later and the tubes left to incubate at 4°C overnight. Bound and free fractions were separated by the addition of a charcoal-dextran solution followed by centrifugation at 1700 g for 15 min (Garnier *et al.*, 1978). The supernatant was decanted into scintillation vials, a single phase scintillant was added and the radioactivity on the vials was measured in a liquid scintillation counter. The scintillant comprised two parts of toluene containing 3 g *p*-terphenyl l $^{-1}$ (Sigma Chemical Co.) and 0.1 g dimethyl POPOP l $^{-1}$ (Ajax Chemicals, Sydney), added to one part of detergent (Teric 70, ICI Ltd, Melbourne).

The limit of assay sensitivity ($\pm$ SEM) defined as the concentration of testosterone corresponding to a count 2 SDs above that for the zero standard was 0.08 ± 0.01 ng ml $^{-1}$, mean nonspecific binding was $1.99 \pm 17\%$, mean zero binding was $49.0 \pm 1.4\%$ and mean binding of a hexane blank was $49.4 \pm 2.47\%$. Each assay contained six replicates of three quality controls, two of which were animal plasmas (mean concentration 0.38 and 2.89 ng ml $^{-1}$, respectively) and one of which had a known concentration of 8.19 ng ml $^{-1}$. The mean intra-assay coefficients for the quality controls were 16.9, 9.4 and 8.1%, and the mean interassay coefficients of variation were 12.7, 7.2 and 6.7%, respectively. For the quality control containing 8.19 ng ml $^{-1}$ the mean recovery was 7.99 ± 0.18 ng ml $^{-1}$. Serial dilution of a sample containing a high concentration of testosterone produced a displacement curve parallel to the standard curve, indicating that the assay was accurate over the range used.

Statistical analysis

LH and testosterone profiles were subjected to pulse analysis using the TURBOPULSAR program (R. Lazarus and D. J. Handelsman, unpublished) based upon an enhanced version of the Pulsar algorithm (Merriam and Wachter, 1982), which enables asymmetrical weighting of deviations from the smoothed baseline. Output from TURBOPULSAR (pulse frequency, pulse amplitude and mean concentration) was analysed by repeated measures analysis of variance with nutrition type as the 'between bucks' factor, and month ($n = 8$) and exposure to oestrous females ($n = 2$) as 'within buck' factors. Data transformations, where indicated, did not influence the conclusions of the analysis; the analysis of untransformed data is therefore presented for ease of interpretation. All analyses were carried out using the SYSTAT 5.1 statistical program (Wilkinson, 1990). Mean separation was carried out using the appropriate linear contrasts within SYSTAT.

Fig. 1. Mean ($\pm$ SEM) monthly liveweight in 3-year-old cashmere bucks fed a low quality diet of pasture hay ($\circ$, $n = 6$) or a high quality diet of pelleted lucerne ($\bullet$, $n = 6$) *ad libitum* for 16 months. Means are based on weekly measurements.

Results

Liveweight

The dietary treatments induced large changes in liveweight ($P < 0.001$), with the effects of month of measurement and the interaction between diet and month also being significant ($P < 0.001$) (Fig. 1). A detailed examination of these effects is provided by Walkden-Brown *et al.* (1994).

LH and testosterone secretion

A summary of the analysis of variance for each of the major secretory variables of these hormones is presented in Table 1. LH and testosterone secretion were significantly influenced by time of year, diet and exposure to oestrous does. For all secretory variables there was a seasonal pattern of change that was little influenced by dietary treatment. The introduction of oestrous does induced major changes in the secretion of both hormones, with the changes depending on the time of year and dietary treatment. The main effects of diet and exposure to oestrous females are shown in Table 2 and Fig. 2, in which the data for all months are pooled, while interactions between these effects and time of year are detailed below and shown in Fig. 3. Representative profiles of LH and testosterone secretion throughout the year for an individual buck from each nutritional treatment are shown in Fig. 4.

LH pulse frequency. In the absence of oestrous does the frequency of LH pulses was influenced by time of year ($P < 0.05$) but not diet, with high frequencies during late summer and autumn and low frequencies during winter and spring (Figs 3 and 4). Exposure to oestrous does increased LH pulse frequency ($P < 0.001$) but the increase depended on both diet and season, and was greatest in bucks on a high quality diet in February (Figs 3, 4). In bucks fed a low quality diet, oestrous does induced a significant increase only in June ($P < 0.05$). The LH response to oestrous does was usually rapid

Table 1. Summary of analysis of variance for LH and testosterone pulse frequency, pulse amplitude and mean concentrations in mature cashmere goat bucks fed two diets and exposed to oestrous does every second month for 16 months

Source	d.f.	Variable ^a					
		LH pulses per 8 h		Mean LH (ng ml ⁻¹)		LH pulse amplitude (ng ml ⁻¹)	
		MS	F	MS	F	MS	F
Diet (D)	1	64.17	5.84*	58.48	2.19	116.32	7.11*
Error ₁ [Bucks] [B]	10	10.98	6.91***	26.71	12.54***	16.36	2.82**
Month (M)	7	23.64	10.51***	21.91	6.17***	188.43	15.86***
M × D	7	3.96	1.76	2.12	0.60	22.67	1.91
Error ₂ [M × B]	70	2.25		3.55		11.88	
Oestrous does (O)	1	68.88	37.72***	91.11	16.02**	35.26	3.63
O × D	1	24.80	13.58**	44.83	7.88*	37.81	3.90
Error ₃ [O × B]	10	1.83		5.69		9.71	
M × O	7	4.52	2.84*	5.92	2.77*	16.10	2.77*
M × O × D	7	3.08	1.94	3.51	1.64	16.76	2.89*
Error ₄ [M × O × B]	70	1.59		2.13		5.80	
		Testosterone pulses per 8 h		Mean testosterone (ng ml ⁻¹)		Testosterone pulse amplitude (ng ml ⁻¹)	
Diet (D)	1	6.10	1.36	203.2	5.89*	123.41	3.68
Error ₁ [Bucks] [B]	10	4.48	4.23***	34.49	11.98***	33.51	7.50***
Month (M)	6	16.32	13.77***	124.20	23.66*	86.36	11.29*
M × D	6	5.29	4.47***	24.06	4.58**	11.86	1.55
Error ₂ [M × B]	60	1.19		5.25		7.65	
Oestrous does (O)	1	42.0	21.00***	190.86	29.60***	31.44	4.97*
O × D	1	13.71	6.86*	44.32	6.87*	1.79	0.28
Error ₃ [O × B]	10	2.0		6.45		6.33	
M × O	6	2.19	2.07	13.78	4.80***	1.95	0.44
M × O × D	6	1.10	1.04	7.13	2.48*	8.13	1.82
Error ₄ [M × O × B]	60	1.06		2.88		4.47	

^aBlood was sampled every 20 min for 8 h before and 8 h during exposure to oestrous does on seven (testosterone) or eight (LH) occasions at two-month intervals. Bucks were fed a low quality diet of pasture hay ($n = 6$) or a high quality diet of pelleted lucerne ($n = 6$), *ad libitum*.

Significant F values : * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

MS: mean square; F: F value; d.f.: degrees of freedom.

Table 2. Main effects of oestrous does and diet on the secretion of LH and testosterone in mature Australian cashmere bucks over a 16-month period

Diet ^a	Hormone parameter	LH		Testosterone	
		Before does ^b	With does ^b	Before does ^b	With does ^b
Low	Pulse frequency (pulses (8 h) ⁻¹)	1.0 ± 0.21 ^{cy}	1.5 ± 0.25 ^{cy}	2.2 ± 0.23 ^{cy}	2.7 ± 0.22 ^{cy}
High	Pulse frequency (pulses (8 h) ⁻¹)	1.4 ± 0.30 ^{cy}	3.3 ± 0.58 ^{dz}	2.1 ± 0.19 ^{cy}	3.6 ± 0.42 ^{dy}
Low	Pulse amplitude (ng ml ⁻¹)	4.8 ± 1.30 ^{cy}	5.6 ± 0.83 ^{cy}	3.2 ± 0.55 ^{cy}	4.3 ± 0.45 ^{cy}
High	Pulse amplitude (ng ml ⁻¹)	6.8 ± 0.70 ^{cy}	7.8 ± 0.92 ^{cz}	5.1 ± 0.81 ^{cy}	5.7 ± 0.91 ^{cy}
Low	Mean concentration (ng ml ⁻¹)	2.8 ± 0.47 ^{cy}	3.3 ± 0.52 ^{cy}	2.2 ± 0.24 ^{cy}	3.3 ± 0.42 ^{dy}
High	Mean concentration (ng ml ⁻¹)	3.0 ± 0.32 ^{cy}	5.3 ± 0.87 ^{dy}	3.3 ± 0.58 ^{cy}	6.5 ± 1.18 ^{dz}

Values are means ± SEM.

^aLow quality diet of pasture hay ($n = 6$) or a high quality diet of pelleted lucerne ($n = 6$), *ad libitum*.

^bBlood was sampled every 20 min for 8 h before and 8 h during exposure to oestrous does on seven (testosterone) or eight (LH) occasions at two-month intervals.

^cMeans within a row and hormone not sharing a common superscript are significantly different ($P < 0.05$).

^zMeans within a column and hormone parameter not sharing a common superscript are significantly different ($P < 0.05$).

(increase within 10 min) and sustained over the 8 h sampling period (Fig. 2). An effect of diet on LH pulse frequency was evident only during exposure to does with bucks fed a high

quality diet having significantly greater pulse frequencies than bucks fed a low quality diet, in December, February and October of the second year ($P < 0.05$) (Fig. 3). Variation

Fig. 2. Effect of oestrous does and diet on mean ($\pm$ SEM) plasma (a) LH and (b) testosterone concentration in mature cashmere bucks fed either a low quality diet of pasture hay ($\circ$, $n = 6$) or a high quality diet of pelleted lucerne ($\bullet$, $n = 6$) *ad libitum* for 16 months. Blood samples were collected at intervals of 20 min between 08.00 and 16.00 h on subsequent days on eight occasions at 2-month intervals. A doe in oestrus was introduced into each buck pen following the 08.00 h blood sample on the second day and left with the buck until blood sampling ceased. Data for all sampling periods are pooled.

between bucks within treatments was significant ($P < 0.001$), with individual means for LH pulse frequency across all sampling periods ranging from 0.4 ± 0.19 to 1.8 ± 0.36 pulses per 8 h in bucks on the low quality diet, and from 0.6 ± 0.21 to 3.5 ± 0.68 in bucks on the high quality diet.

LH pulse amplitude. LH pulse amplitude was strongly influenced by time of year ($P < 0.001$), with high amplitudes in spring and summer and low amplitudes during autumn and winter (Fig. 3). Diet induced seasonally dependent changes: bucks fed a high quality diet had significantly greater pulse amplitudes than did bucks fed a low quality diet during April, May and June, irrespective of the presence of oestrous does ($P < 0.05$). Oestrous does had little overall effect on LH pulse amplitude, although a significant increase was observed following their introduction to bucks on a high quality diet in August 1989 ($P < 0.05$). Variation between bucks within treatments was significant ($P < 0.01$), with mean pulse amplitude across all sampling periods ranging from 3.0 ± 0.6 to 9.5 ± 1.7 ng ml⁻¹ in bucks on the low quality diet, and from 5.5 ± 1.9 to 9.6 ± 1.2 ng ml⁻¹ in bucks fed the high quality diet.

Mean LH concentration. The mean LH concentration was strongly correlated with LH pulse frequency ($r = 0.84$, $n = 180$, $P < 0.001$) and weakly correlated with pulse amplitude ($r = 0.39$, $n = 131$, $P < 0.01$) across all bucks and sampling periods. In the absence of oestrous does there was a significant effect of time of year ($P < 0.001$) but not diet, with high concentrations during summer and autumn and low concentrations in winter (Fig. 3). Exposure to oestrous does induced season- and diet-dependent increases in LH concentration ($P < 0.001$), with non-significant increases in bucks on a low quality diet and much larger increases in bucks on a high quality diet ($P < 0.01$) – particularly during the summer months (Figs 2, 3). Bucks within treatments were a significant source of variation ($P < 0.001$) with individual means across all

sampling periods ranging from 1.7 ± 0.09 to 4.5 ± 0.18 ng ml⁻¹ in bucks on the low quality diet, and 2.1 ± 0.11 to 5.6 ± 1.0 ng ml⁻¹ in bucks on the high quality diet.

Testosterone pulse frequency. Well-defined pulses of testosterone followed those of LH by an average of 45 ± 1.5 min (Fig. 4), but the correlation between the number of pulses of the two hormones across all animals and sampling periods was only moderate ($r = 0.67$, $n = 180$, $P < 0.001$). In the absence of oestrous does, there was a significant effect of time of year ($P < 0.001$) but not of diet, with high pulse frequencies in autumn, and low frequencies in late winter (Figs 3, 4). Exposure to oestrous does increased the frequency of testosterone pulses ($P < 0.001$) in a diet- and season-dependent way; the increase was greatest in bucks fed a high quality diet between December and June (Figs 3, 4). In bucks fed on a low quality diet oestrous does induced a significant increase only in June ($P < 0.05$). An effect of diet was evident only during exposure to oestrous does, with bucks on high quality diet exhibiting significantly greater pulse frequencies than did bucks on low quality diet in December and February ($P < 0.05$). Bucks within treatments differed significantly ($P < 0.001$), with individual means for testosterone pulse frequency across all sampling periods ranging from 1.7 ± 0.28 to 3.0 ± 0.38 pulses per 8 h for bucks on the low quality diet, and from 1.6 ± 0.27 to 3.1 ± 0.65 for bucks on the high quality diet.

Testosterone pulse amplitude. Testosterone pulse amplitude was significantly influenced by time of year ($P < 0.001$) and exposure to oestrous does ($P < 0.05$), with no interaction between these effects (Figs 3, 4). In both treatments, pulse amplitudes were high in late summer and autumn and lowest in late winter. Despite a weak positive association between LH and testosterone pulse amplitude overall ($r = 0.39$, $n = 126$, $P < 0.001$), there was a clear seasonal change in the relationship between the two, with high-amplitude LH pulses inducing

Fig. 3. Effect of oestrous does and diet on mean ($\pm$ SEM) (a) LH and (b) testosterone pulse frequency, pulse amplitude of (c) LH and (d) testosterone and mean plasma concentrations of (e) LH and (f) testosterone in mature cashmere bucks fed either a low quality diet of pasture hay ($n = 6$) or a high quality diet of pelleted lucerne ($n = 6$) *ad libitum* for 16 months. Blood samples were collected at intervals of 20 min between 08.00 and 16.00 h on subsequent days on eight occasions at intervals of 2 months. A doe in oestrus was introduced into each buck pen following the 08.00 h blood sample on the second day and left with the buck until blood sampling ceased. Groups are as follows: low quality diet before does ($\square$); low quality diet with does ($\blacksquare$); high quality diet before does ($\circ$) and high quality diet with does ($\bullet$).

low-amplitude testosterone pulses in winter and spring, and low-amplitude LH pulses inducing high-amplitude testosterone pulses in late summer and autumn (Figs 4, 5). Exposure to oestrous does led to significant increases in pulse amplitude in bucks on low quality diets in June and bucks on high quality diets in August of the second year ($P < 0.05$). Pulse amplitude varied significantly between bucks within treatments ($P < 0.001$), with individual means across all sampling periods ranging from 1.7 ± 0.31 to 4.1 ± 0.75 ng ml $^{-1}$ for bucks on low quality diets and from 2.3 ± 0.61 to 5.7 ± 0.76 ng ml $^{-1}$ in bucks on high quality diets.

Mean testosterone concentration. Mean testosterone concentration was closely associated with both testosterone pulse frequency ($r = 0.76$, $n = 180$, $P < 0.001$) and pulse amplitude ($r = 0.65$, $n = 173$, $P < 0.001$) across all bucks and sampling

periods. In the absence of oestrous does, testosterone concentrations varied significantly with time of year ($P < 0.001$) but not diet ($P = 0.1$), being highest in autumn and lowest in winter (Fig. 3). Exposure to oestrous does increased testosterone concentrations ($P < 0.001$) (Fig. 2), but this effect was dependent upon both time of year and diet with maximal responses in bucks on the high quality diet in February (Fig. 3). Oestrous does induced significant increases in testosterone concentrations in February, June and August 1989 in bucks on high quality diet, and in June in bucks on low quality diet ($P < 0.05$). Diet influenced testosterone concentration significantly only following exposure to oestrous does (Table 2, Fig. 2), with bucks fed the high quality diet having significantly higher concentrations than those fed the low quality diet overall and in December and February ($P < 0.05$). Bucks within treatments were a significant source of variation in mean testosterone

Fig. 4. Representative profiles of LH (●) and testosterone (○) secretion throughout the year in an individual cashmere buck fed (a) a low quality diet of pasture hay and (b) one fed a high quality diet of pelleted lucerne *ad libitum* for 16 months. Blood samples were collected at intervals of 20 min between 08.00 and 16.00 h on subsequent days. On the second day a doe in oestrus was introduced into each buck pen following the 08.00 h blood sample.

concentrations ($P < 0.001$), with individual means across all sampling periods ranging from 1.8 ± 0.33 to 3.2 ± 0.89 ng ml^{-1} in bucks fed a low quality diet and 1.1 ± 0.15 to 6.9 ± 1.6 ng ml^{-1} in bucks fed a high quality diet.

Discussion

The results demonstrate that (i) Australian cashmere bucks exhibit a seasonal cycle of LH and testosterone secretion,

(ii) exposure to oestrous females induces a rapid increase in the secretion of LH and therefore testosterone, and the magnitude of this increase depends upon both the time of year and the nutritional status of the buck; and (iii) differences in the nutritional status of bucks may induce changes in the secretion of LH and testosterone that are evident only following exposure to oestrous females.

The seasonal changes in LH and testosterone secretion observed in bucks before exposure to oestrous does are

Fig. 5. Seasonal change in the relationship between LH (■) and testosterone (▨) pulse amplitude in mature cashmere bucks. Means ($\pm$ SEM) include bucks on low quality and high quality diets before and after exposure to oestrous does.

broadly similar to those in other studies (Pygmy goats, Muduuli *et al.*, 1979; Howland *et al.*, 1985; Alpine and Saanen goats, Delgadillo and Chemineau, 1992), with seasonal maxima in pulse frequency and plasma concentrations in late summer and autumn and minima in late winter and spring. It is possible that the seasonal changes observed were due to the intermittent exposure to oestrous does, but this is unlikely since the seasonal pattern of change in gonadotrophin concentrations was similar to that seen in cashmere bucks and bucks of other breeds when totally isolated from does (Delgadillo and Chemineau, 1992; Walkden-Brown *et al.*, 1992). It is far more likely that the changes are driven by changing photoperiod (Delgadillo and Chemineau, 1992), and mediated by changing responsiveness of the hypothalamic GnRH pulse generator to negative feedback from gonadal steroids (Walkden-Brown *et al.*, 1992). LH pulse amplitude showed a seasonal pattern almost the inverse of that of LH pulse frequency, with minimal amplitudes in autumn and early winter and maxima in spring. The spring maximum in pulse amplitude is in agreement with the observations of Muduuli *et al.* (1979) in the Pygmy goat but is somewhat earlier than the summer maximum reported by Delgadillo and Chemineau (1992) in more seasonal European goat bucks.

In contrast to LH, testosterone pulse amplitude was highest in autumn when LH and testosterone pulse frequency was maximal, with the small LH pulses characteristic of this period inducing large testosterone pulses, while the much larger LH pulses of the spring and summer induce testosterone pulses of only moderate amplitude. It is probable that this seasonal variation in testicular responsiveness to LH is associated with seasonal changes in LH receptor populations in the testis as is the case in the ram (Barenton and Pelletier, 1983). While the change in testicular responsiveness to LH resulted in a weak association between LH and testosterone pulse amplitudes over the whole experiment, there was evidence that within sampling periods the amplitude of an individual LH pulse was positively related to the amplitude of the resultant pulse of testosterone.

The effects of diet on unstimulated bucks were restricted to an increase in LH pulse amplitude in the bucks during autumn

and early winter, and a non-significant trend towards a higher LH pulse frequency in the same bucks during spring and summer. The absence of significant effects on LH and testosterone pulse frequencies and mean concentrations is surprising given the well-documented inhibitory effects of undernutrition on LH secretion in sheep (Haresign, 1981; Foster *et al.*, 1988, 1989; Martin *et al.*, 1989; Thomas *et al.*, 1990), cattle (Imakawa *et al.*, 1986), pigs (Britt *et al.*, 1988) and rats (Howland, 1972). Improving nutrition from maintenance to supra-maintenance has also been shown to increase LH secretion in Merino rams in the short term (< 4 weeks) but not the longer term (Ritar *et al.*, 1984; Martin *et al.*, 1987, 1989). The lack of a nutritional effect on LH secretion in unstimulated bucks in the present experiment may reflect the mild undernutrition imposed on bucks on the low quality diet (maintenance at moderate condition score rather than submaintenance), or it may be that acute increases in LH secretion induced by the improved diet were not detected by the sampling regimen used. What the current findings do suggest is that the effects of suboptimal nutrition on the gonadotrophic axis may be subtle enough to be undetectable in the non-stimulated animal, but that when the axis is challenged, its capacity to respond may be impaired.

Exposure to oestrous does increased LH and testosterone concentrations but the response depended on both season and diet. When this endocrine response occurred, it was rapid and generally sustained over the 8 h sampling period. This finding is consistent with the observation of Sanford *et al.* (1974) that the duration of the LH and testosterone response in rams exposed to a single oestrous ewe is approximately 12 h. In most responsive cases, an increase in LH concentration was evident at the first sample taken after the introduction of the female; however, there were cases when a clear response, demonstrated by a cluster of LH pulses, was delayed for up to 220 min after the introduction of the oestrous female. While the response was usually sustained throughout the exposure to oestrous females, in a small number of cases LH concentrations surged dramatically before declining 3–4 h after introduction. In a similarly small number of cases LH concentration was still increasing 8 h after exposure. Whether these variations represent differences in male responsiveness or in the intensity of the female stimulus is not known.

There was a clear seasonal component in responsiveness with increases in LH secretion evident in summer, autumn and early winter but not during late winter and spring when photoperiod inhibition of the gonadotrophic axis is at a maximum (Delgadillo and Chemineau, 1992). This is in agreement with the findings of Howland *et al.* (1985) in Pygmy goats, and suggests that the female stimulus is unable to overcome photoperiod inhibition when it is at maximal levels. The provision of a high quality diet did not assist in overcoming the seasonal nadir in the activity of the gonadotrophic axis, indicating that social and nutritional stimulation alone or in combination were ineffective during this period. In this regard the cashmere buck resembles the doe, in which exposure to bucks from mid-spring to mid-summer generally fails to induce an ovulatory response (Cameron and Batt, 1989; Restall, 1992), although exceptions have been reported (Walkden-Brown, 1993a). It is possible that the seasonal variation in the response to oestrous does was due more to seasonal changes in female behaviour than to variation in male responsiveness. However,

there was no evidence of seasonal variation in female behaviour, and the standardized oestrus induction method, using ovariectomized does, was designed to minimize such variation.

Diet induced pronounced effects on the response to oestrous does during the period when photoperiodic inhibition of the gonadotrophic axis was waning or at its seasonal minimum. In bucks on the high quality diet, responses to oestrous does were first evident in December, reached a maximum in February and continued through to August, while in bucks on the low quality diet, responses commenced in February, peaked in June and were absent by August. Thus, the high quality diet had the effect of advancing the onset, and extending the duration, of responsiveness to oestrous does. The level of nutrition has been shown to influence the timing or the duration of seasonal reproductive cycles in ewes (Smith, 1965; Hulet *et al.*, 1985; Oldham *et al.*, 1990) and rams (Masters and Fels, 1984), as well as the timing and duration of responsiveness to the 'male effect' in ewes (Wright *et al.*, 1990). This modulation of seasonal reproductive phenomena suggests that nutrition may act by modulating hypothalamic responsiveness to negative steroid feedback, the primary mechanism by which photoperiod influences reproduction in sheep (Legan *et al.*, 1979) and goats (Chemineau *et al.*, 1988; Walkden-Brown *et al.*, 1992). Indeed, there is considerable evidence that the effects of acute changes in nutritional status on LH pulse frequency are at least partly mediated by this mechanism in rats (Howland and Ibrahim, 1973; Piacsek, 1985), cattle (Imakawa *et al.*, 1986) and sheep (Rhind *et al.*, 1986; Tjondronegoro *et al.*, 1991), although steroid-independent effects have also been clearly demonstrated in each of these species (Dong *et al.*, 1990; Tatman *et al.*, 1990; Thomas *et al.*, 1990; Roberson *et al.*, 1991).

Apart from influencing the onset and duration of responsiveness to oestrous does, diet significantly increased the magnitude of the LH and testosterone response in bucks on the high quality diet. This occurred without major effects on LH and testosterone secretion in the unstimulated animal, suggesting that nutritional status may play a specific role in regulating the responsiveness of the gonadotrophic axis to acute environmental stimuli such as exposure to the opposite sex. This role may differ from its effects on gonadotrophin secretion in the unstimulated animal. The effect of oestrous females on the GnRH pulse generator appears to be mediated by a direct stimulatory mechanism rather than by a simple release from photoperiodic and nutritional inhibition, since the extraordinary secretory rates of LH induced by this stimulus under optimal conditions far exceed the rates achieved when both nutrition and photoperiod are optimal but the female stimulus is absent. Support for a direct role is also provided by the finding that ovariectomized ewes will respond to the introduction of rams with an increase in LH pulse frequency in the absence of steroid implants (Martin *et al.*, 1986).

It is interesting that maximal responses to oestrous does occurred in summer for bucks on the high diet and in winter for bucks on the low diet rather than in mid-autumn, the time when photoperiodic inhibition could be expected to be at its seasonal minimum. Bucks on both diets lost weight throughout autumn and it is possible that this may have imposed additional inhibitory tone on the gonadotrophic axis and attenuated responses to the female stimulus. Support for this concept is provided by the finding of Roberson *et al.* (1991) that the effect

of nutrition on gonadotrophin secretion in ovariectomized heifers depends upon the direction of liveweight change, rather than absolute liveweight. In other studies on the female effect (Yarney and Sanford, 1983; Howland *et al.*, 1985; Schanbacher *et al.*, 1987) the absence of a response to oestrous females during this period has led to the postulate that responses do not occur because the gonadotrophic axis is already maximally stimulated at this time of the year. This is an unlikely explanation for the present results given that exposure to oestrous does in February induced an LH pulse frequency of 7.2 per 8 h in bucks on high quality diet indicating that the capacity of the GnRH pulse generator to be stimulated is extremely high, certainly far greater than that observed in stimulated or unstimulated bucks during autumn.

One consequence of exposing bucks to oestrous females was an increase in testosterone pulse amplitude independent of changes in LH pulse amplitude, season or diet. It is difficult to explain this increase in testosterone pulse amplitude in the absence of a similar effect on LH pulse amplitude. However, it is possible that there were changes in LH pulse amplitude that were not detected. The duration of a pulse of LH is shorter than that of testosterone, and the error associated with measuring pulse amplitudes from samples collected at intervals of 20 min would be greater for LH than for testosterone, possibly masking an increase in pulse amplitude.

There was significant variation between individual males within treatments, for all measures of LH and testosterone secretion. Two animals, buck 5291 on the low quality diet, and buck 5118 on the high quality diet, exhibited zero or low LH and testosterone pulse frequency throughout the year without significant seasonal variation, and did not respond to oestrous females with an increase in LH and testosterone secretion, apart from a non-significant increase in April, which was well below that observed in other bucks in their respective treatments. One buck exhibited prominent homosexual behaviour although he also mated females successfully. The extreme individual variation observed is probably due to the heterogeneous nature of the base population, which comprised domesticated feral goats (Restall and Pattie, 1989).

Although exposure to oestrous females is known to increase LH secretion in other breeds of goat (Howland *et al.*, 1985) and mammalian species including cattle (Katagole *et al.*, 1971; Lunstra *et al.*, 1989), sheep (Sanford *et al.*, 1974; Schanbacher *et al.*, 1987; Gonzalez *et al.*, 1988a, b) and mice (Bronson and Desjardins, 1982), the functional significance of the female effect has yet to be determined. It is possible that the associated increase in testosterone secretion leads to behavioural changes that improve mating success rates, although reproductive behaviour does not appear to be acutely sensitive to testosterone concentrations (Knight, 1973; Mattner and Braden, 1975; Lincoln and Davidson, 1977). Nevertheless, exposure to oestrous females clearly enhances the ability of rams (Knight, 1985) and goat bucks (Walkden-Brown *et al.*, 1993b) to induce ovulation in anovulatory females. Walkden-Brown *et al.* (1993b) suggested that the female effect is one component of a self-reinforcing cycle of stimulation that may be initiated by either sex. Such a cycle may be of considerable importance in initiating and synchronizing reproductive activities in breeds and species such as the cashmere goat, which exhibit a considerable degree of reproductive opportunism (Harrington, 1982).

In conclusion the current results confirm the original hypotheses that there is a seasonal cycle in LH and testosterone secretion in mature cashmere bucks, and that nutrition and oestrous females are powerful modulators of the secretion of these hormones in a seasonally dependent way.

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