Ovulation rate and the concentrations of gonadotrophic and metabolic hormones in ewes fed lupin grain

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An experiment in which a lupin grain supplement was fed to ewes ($n = 11$) over days 2–13 of the oestrous cycle was carried out. A group of 12 ewes was used as a control and not fed the supplement. Plasma concentrations of LH and GH (20 min intervals) and FSH, insulin and prolactin (hourly intervals) were determined in plasma samples collected every 20 min over 24 h on day 11 of the oestrous cycle. The changes were related to increases in ovulation rate. Ovulation rate was increased (2.5 ± 0.2 versus 1.9 ± 0.2 for lupin-supplemented and control groups, respectively; $P = 0.073$) in the group that received the lupin supplement, but this increase was not associated with significant changes in either LH or FSH concentrations on day 11 of the oestrous cycle. Lupin supplementation had significant effects on the plasma concentrations of prolactin, GH and insulin. There was a transient increase ($P < 0.05$) in the concentration of prolactin 4–8 h after feeding, whereas insulin concentrations were increased immediately after feeding ($P < 0.02$) and were still high 24 h later ($P < 0.02$). Growth hormone concentrations were reduced in ewes fed with lupin grain ($P < 0.001$). These metabolic responses initiated by feeding a high energy and protein supplement such as lupin grain may be related to changes in ovulation rate. In particular, the sustained increases in insulin concentrations suggest that an increased supply of glucose to the follicle mediates nutritionally stimulated increases in ovulation rate.

Introduction

Lupin (*Lupinus angustifolius*) is a legume high in digestible energy and protein that, when fed to ewes, increases their ovulation rate (Lightfoot and Marshall, 1974; Knight *et al.*, 1975). Since these early studies, there have been numerous other reports of increased ovulation rate following lupin grain supplementation (for a review see Smith and Stewart, 1990). Increases in ovulation rate occur within as little as 6 days after the start of feeding lupin grain (Lindsay, 1976) and the critical period for lupin grain supplementation to obtain increases in ovulation rate is 4–6 days before luteolysis (Nottle *et al.*, 1986; Stewart and Oldham, 1986). The physiological mechanism(s) leading to this increase is not clearly understood.

Ewes given a lupin supplement have higher mean plasma concentrations of FSH before oestrus (Brien *et al.*, 1976; Knight *et al.*, 1981). Similarly, concentrations of FSH in plasma of ewes fed a high protein diet that increases ovulation rate are higher on days 2, 3, 4 and 6 before oestrus (Smith, 1988). Plasma FSH concentrations increased within a day in oestradiol-implanted, ovariectomized ewes fed lupin grain for 7 days, compared with similar ewes fed wheaten chaff alone (Nottle *et al.*, 1987), suggesting an effect on the hypothalamo–pituitary axis. Since exogenous FSH increases the ovulation rate (McNatty *et al.*,

1985), it is suggested that increases in ovulation rate following lupin grain supplementation involve increases in the secretion of FSH at a time in the oestrous cycle when exogenous FSH increases ovulation rate (McNatty *et al.*, 1985).

However, there are reports that suggest the effect of lupin grain supplementation on ovulation rate does not involve changes in gonadotrophin concentrations. Lupin grain supplements have been shown to increase ovulation rate with no difference in the pattern of release of LH or FSH in either the luteal phase or the follicular phase of the oestrous cycle (Radford *et al.*, 1980; Ritar and Adams, 1988), suggesting that supplementation with lupin grain may also have local effects on ovarian function.

The effect of lupin grain supplementation, whether it acts on the hypothalamo–pituitary axis or directly on the ovary, is likely to be associated with metabolic changes that accompany the increase in nutrient supply. The present experiment was designed to relate the circulating concentrations of gonadotrophins to the metabolic hormones, insulin, prolactin and GH, in ewes fed a basal diet of oaten straw and given a lupin grain supplement over days 2–13 of the oestrous cycle.

Materials and Methods

Animals

Twenty-three Border Leicester × Merino ewes, 5–6 years of age, were used. During the course of the experiment they were

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housed in single pens in an open sided shed and had free access to fresh water at all times.

Experimental design

Day 0 is the day on which straw feeding commenced. Oestrus was synchronized with two injections of 125 µg prostaglandin $F_{2\alpha}$ analogue (PG; Cloprostenol, Estrumate: ICI Pty Ltd, Sydney) on days 5 and 15. Observations were carried out during the luteal phase (day 11 of the oestrous cycle or day 28 after the start of feeding) of the oestrous cycle that followed the second injection of PG. Lupin supplementation was given from day 19 to day 30 (days 2–13 of the oestrous cycle). After the period of lupin supplementation, all ewes were given a third PG injection on day 30. The ovulation rate was determined by endoscopy on day 40.

Diet

The basal diet consisted of hammermilled oaten straw supplemented with minerals. A 10 mm screen was used and the straw was prepared in 400 kg batches. To each batch salts (CaCO_3 : 1.99 kg; Na_2SO_4 : 1.54 kg; MgCO_3 : 0.40 kg; NaCl : 1.99 kg; $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$: 3.98 kg; KCl : 1.33 kg) and trace minerals (H_2SeO_3 : 65.1 mg; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$: 12.2 g; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$: 72.6 mg; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$: 2.30 g; KI : 51.9 mg; $(\text{NH}_4)_2\text{SO}_4\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$: 55.6 g) were added. The salts, straw and minerals were mixed for 30 min before they were dispensed into bags and stored. Lupin grain (*Lupinus angustifolius*, cv Uniwhite) was obtained from a commercial source.

The oaten straw (2.3% protein on a dry matter basis) had a dry matter content of 91.6% with a gross energy of 20.8 MJ kg^{-1} (Leche *et al.*, 1982). The dry matter digestibility and the protein digestibility of the straw diet were estimated in a group of six ewes (Downing, 1994) at $41.1 \pm 1.5\%$ and $44.2 \pm 2.4\%$ (mean $\pm$ SEM), respectively. Lupins (33.6% protein on a dry matter basis) have a dry matter content of 91.1% and a gross energy value of 20.1 MJ kg^{-1} (Margan, 1994). Margan (1994) reported the energy digestibility and the protein digestibility of lupins as $63.1 \pm 1\%$ and $75 \pm 1\%$, respectively.

Feeding

At the start of the experimental period (day 0) all ewes were fed the oaten straw diet at a rate of 800 g day^{-1} and individual feed intakes were measured. On day 19 (day 2 of the oestrous cycle and 4 days after the second PG injection), eleven of the ewes were each offered 150 g of lupin grain and then on days 3–5 of the oestrous cycle they were offered 250, 400 and 600 g, respectively. From day 6 to day 13 of the oestrous cycle, the ewes were each offered 750 g of lupin grain daily. The lupin supplement was given at 09:00 h each day and any residual was removed at 15:00 h. The ewes were then offered 800 g of straw. During the treatment period, the 12 control ewes were offered 800 g of the straw at 09:00 h daily. Over the period of lupin supplementation, straw residues were also removed daily and weighed.

Live mass

The live masses of all ewes were determined before feeding, on days 6, 12, 23, 33 and 40.

Cannulation

On day 27 (that is day 10 of the oestrous cycle and 12 days after the second PG injection) all ewes were fitted with one indwelling jugular venous catheter.

Blood sampling

Blood samples (10 ml) were taken by venepuncture 2–3 times a week from day 0 for estimation of plasma progesterone concentrations. On the day following cannulation, 3 ml blood samples were collected every 20 min for 24 h starting at 10:00 h. The blood was centrifuged (20 min at 4°C and 1000 g) and the plasma stored at -20°C .

Assays

Progesterone (Campbell *et al.*, 1990), LH (Scaramuzzi *et al.*, 1970) and FSH (Campbell *et al.*, 1991) were determined using radioimmunoassays. Progesterone concentrations were determined in the samples collected three times a week. The intra-assay coefficient of variation was 8.5%; the interassay coefficient of variation was 11.9%; and the sensitivity (defined as the concentration of hormone that produces binding that is 2 SD less than the zero binding) of the assay was 0.05 ng ml^{-1} . All plasma samples collected over the 24 h of intensive sampling were assayed for LH. The intra-assay coefficient of variation was 9.4%; the interassay coefficient of variation was 11.9%; and the sensitivity of the assay was 0.2 ng ml^{-1} . Samples collected on the hour were assayed for FSH. The intra-assay coefficient of variation was 5.8%; the interassay coefficient of variation was 10.9%; and the sensitivity of the assay was 0.07 ng ml^{-1} .

The concentrations of GH, prolactin and insulin (Downing, 1994) were also determined by radioimmunoassay.

The samples collected at intervals of 20 min were assayed for their GH concentration using the following method. The antiserum (NIDDK-anti-oGH-2) used in the GH assay was raised in a rabbit, diluted 1:90 in phosphate buffer and stored in aliquots of 100 and 200 µl at -20°C . When required, antiserum was diluted in phosphate buffer, to give an initial dilution of 1:30 000. All standards and quality controls were prepared using the GH standard reference preparation (NIDDK-oGH-I-4) in GH-free plasma collected from hypophysectomized sheep. The oGH reference preparation was iodinated by the chloramine T method. For use in the assay, the label was diluted in phosphate buffer with EDTA to give 12 000–15 000 c.p.m. in 50 µl. Standard, quality control (QC) or samples of plasma (100 µl) were diluted with 300 µl of phosphate buffer and 50 µl of the antiserum solution containing normal rabbit serum (1:600) was added. After overnight incubation at 4°C, 50 µl [^{125}I]-labelled oGH was added. The tubes were incubated for a further 2 days at 4°C and then 50 µl of donkey anti-rabbit gamma globulin serum was added. The

assay was left overnight at 4°C. The next morning the tubes were centrifuged at 4°C (60 min at 1900 g), the supernatant aspirated and the pellet (bound fraction) counted in a gamma spectrophotometer. The sensitivity of the assay ($n = 14$) was estimated as 0.45 ng ml⁻¹. The intra-assay and interassay CVs estimated using 3 QCs containing low, medium and high concentrations of GH were: low (2.5 ng ml⁻¹): 8.6% and 10.3%; medium (6.7 ng ml⁻¹): 9.2% and 9.9%; and high (13.1 ng ml⁻¹): 7.5% and 7.9%, respectively.

Samples collected on the hour were assayed for their prolactin concentration using the following method. The antiserum (NIDDK-anti-oPRL-2) used in the prolactin assay was raised in a rabbit, diluted 1:600 in phosphate buffer and stored in aliquots of 100–200 µl at -20°C. When required, sufficient antiserum was diluted in phosphate buffer to give an initial dilution of 1:300 000. All prolactin standard reference preparation (NIDDK-oPRL-I-2; AFP-7150B) and the QC samples were prepared in prolactin-free plasma from hypophysectomized sheep. The prolactin reference preparation was iodinated by the chloramine T method. For use in the assay, the label was diluted in phosphate buffer with EDTA to give 12 000–15 000 c.p.m. in 100 µl. Standard QC or samples of plasma (100 µl) were diluted with 600 µl of phosphate buffer and 100 µl of the antiserum containing normal rabbit serum (1:600) was added to each tube. The assay was incubated at 4°C overnight before adding 100 µl [¹²⁵I]-labelled oprolactin. The assay was incubated for 2 days at 4°C and 100 µl of donkey anti-rabbit gamma globulin serum was then added to each tube. After overnight storage at 4°C, the tubes were centrifuged at 4°C for 60 min at 1900 g. The supernatant was aspirated and the radioactivity in the precipitate determined in a gamma spectrophotometer. The sensitivity of the assay ($n = 15$) was estimated as 1.6 ng ml⁻¹. The intra-assay and interassay CVs estimated using 3 QCs containing low, medium and high concentrations of prolactin were: low (3.2 ng ml⁻¹): 7.0% and 9.8%; medium (27.2 ng ml⁻¹): 7.8% and 9.1%; and high (123 ng ml⁻¹): 10.5% and 15.5%, respectively.

Plasma insulin concentrations were determined in hourly samples between 1 h and 5 h and between 21 h and 24 h after lupin feeding using the following method. The antiserum was raised in a guinea-pig against bovine insulin (PJ 4499; Eli Lilly, Sydney). The crossreactivities with other hormones were: ovine insulin (Sigma, Sydney) 100%, bovine insulin (Novo, Melbourne) 100%, porcine insulin (Novo) 56%, equine insulin (Novo) 63%, bovine pro-insulin 32%, bovine C peptide 0%, ovine GH (NIH-NIDDK-oGH-I-4) 0%, bovine glucagon (Novo) 0% and somatostatin (Peptide Technology, Dee Why, NSW) 0%. The antiserum was diluted (1:100) in phosphate buffer and stored in aliquots of 100–200 µl at -20°C. When required, sufficient antiserum was diluted in phosphate buffer, to give an initial dilution of 1:100 000. Standards of insulin (SPJ 4499; Eli, Lilly) were prepared in insulin-free plasma obtained by mixing plasma from a castrated ram with activated charcoal (10 mg ml⁻¹) overnight. The following day, the charcoal and plasma were separated by centrifugation at 1000 g for 15 min at 4°C. Plasma samples collected from three ewes on different diets were used as internal QCs. Insulin (SPJ 4499; Eli, Lilly) was iodinated by the chloramine T method. For use in the assay, the label was diluted in phosphate buffer with EDTA to give 12 000–15 000 c.p.m. in 100 µl. Standard QC or samples

of plasma (100 µl) were diluted with 500 µl of phosphate buffer and 100 µl of insulin-free plasma (standards only), and then 100 µl of the antiserum containing normal guinea-pig serum (1:600) was added to each tube. The assay was incubated at 4°C overnight before adding 100 µl [¹²⁵I]-labelled insulin. The assay was incubated for 2 days at 4°C and 100 µl of donkey anti-guinea-pig gamma globulin was then added to each tube. The remainder of the insulin assay followed the same procedure as the prolactin assay. The sensitivity of the assay ($n = 6$) was estimated as 0.09 ng ml⁻¹. The intra-assay and interassay CVs estimated using 3 QCs containing low, medium and high concentrations of prolactin were: low (0.5 ng ml⁻¹): 10.9% and 9.6%; medium (1.7 ng ml⁻¹): 5.7% and 5.2%; and high (4.2 ng ml⁻¹): 7.3% and 3.9%, respectively.

For each ewe, a 50 µl aliquot was taken from the hourly plasma samples and these were pooled by animal and assayed to determine IGF-I concentrations (Francis *et al.*, 1989; Speck, 1991). The intra-assay coefficient of variation was 1.0% and the interassay coefficient of variation was 8.9%.

Amino acid analysis

A 10 ml blood sample was taken 23 h after the lupin supplement was given on day 11 of the oestrous cycle and the blood processed for analysis and stored at -20°C. The blood was centrifuged at 1000 g for 15 min at 4°C as soon as possible after collection to avoid transformation of the sulfur-containing amino acids. After centrifugation, 2.4 ml plasma was added to 0.6 ml of a 5-sulfosalicylic acid (AnalaR; BDH Chemicals Ltd, Poole) solution, prepared by dissolving 10 g in 100 ml of distilled water. The mixture was left for 30 min at 4°C and then centrifuged at 5000 g for 20 min. After centrifugation, 1.8 ml of the supernatant was diluted with lithium citrate buffer (pH 2.2), prepared by dissolving 9.49 g tri-lithium citrate (4H₂O), 7.4 g citric acid (H₂O) and 20 ml of a 25% solution of 2,2'-thioethanol in 1 litre of distilled water. The samples were then stored at -20°C until analysed. Amino acids were separated and analysed by ion exchange chromatography on a column of sulfonated polystyrene beads with 10% cross-linkage (Biotronik Type BTC-2710 G58181) in conjunction with an automatic amino acid analyser (Model LC 5001, Biotronik, Munich). The samples were eluted with a lithium citrate buffer. After reaction with ninhydrin, absorbancies were measured. Integration of the peaks was performed by an A/D converter (Nelson 860) connected to a microprocessor (DOT, model 14547 08).

Statistical analyses

Differences in ovulation rate were compared by a chi-squared test of independence using categories of 1, 2 or 3 ovulations. Differences in mean mass were compared by an unpaired Student's *t* test. The LH pulse analysis was carried out using the Munro pulse analysis program (Elsevier, Biosoft, Cambridge). Comparisons of the pulse characteristics between treatment groups were made by an unpaired Student's *t* test. The effects of time and of treatment and their interaction were analysed by a repeated measures analysis of variance (CLR, ANOVA; Clear Lake Research Incorporated, Houston, Texas).

Table 1. The mean ($\pm$ SEM) live mass of ewes fed a basal diet of straw or straw and a lupin grain supplement for days 2–13 of the oestrous cycle

Days relative to start of straw feeding	6	13	23	33	40
Days relative to start of lupin feeding	- 22	- 14	4	14	21
Treatment					
Straw (kg)	63.6 $\pm$ 0.6	61.8 $\pm$ 0.6	59.6 $\pm$ 0.7	57.4 $\pm$ 0.7	55.6 $\pm$ 0.6
Lupin (kg)	63.1 $\pm$ 0.6	61.7 $\pm$ 0.5	60.1 $\pm$ 0.6	61.1 $\pm$ 0.7*	59.8 $\pm$ 0.8*

*Significantly different from those of straw fed ewes ($P < 0.01$).

for feed intake and for the plasma concentrations of FSH, prolactin, GH and insulin. The effect of treatment at given times was tested using a pooled error term. Because of the large within and between animal variation, the analysis of prolactin and GH data was carried out after log transformation of the data.

Results

The plasma progesterone concentrations indicated that all ewes were cyclic in the expected manner and had functioning corpora lutea of the same age at the end of the period of lupin feeding.

Ovulation rate

The ovulation rate of ewes supplemented with lupin grain tended to be higher (2.5 ± 0.2) than that of control fed ewes (1.9 ± 0.2) ($P = 0.073$).

Live mass

Up until 23 days after the start of straw feeding, which included the first 4 days of lupin feeding, there was no significant difference in the mean mass (Table 1). By day 33, 2 days after the end of the lupin feeding, the ewes fed the lupin supplement had a significantly higher mean mass ($P < 0.01$). Ten days after the end of the lupin feeding period, this difference was still apparent.

Feed intake

Over the first 5 days of supplementation, the mean lupin grain intake increased from 98 ± 18 g to 505 ± 46 g. Over the period that the ewes were offered 750 g of lupin grain per day, the mean daily lupin intake was 574 ± 35 g. None of the supplemented ewes consumed all of the 750 g of grain offered on all of the days. Usually ewes consumed all or most of the grain supplement for 2–3 days and then did not consume any, or very little, grain on the next day. After a day of low lupin intake the ewes would usually consume all of the grain for the next 2–3 days.

The intake of straw in the lupin-supplemented ewes tended to be depressed when the ewes were offered 750 g of the lupins day^{-1} (Fig. 1: $P < 0.1$ at days 18 and 20). However, this

Fig. 1. The mean daily straw intake for ewes offered 800 g day^{-1} of straw or straw supplemented with lupin grain. Initially, both groups were fed the straw diet; one group was then fed the straw diet plus a lupin grain supplement ($\bullet$) while the other group continued to be fed straw ($\circ$). Increasing quantities of grain up to 600 g day^{-1} were offered for the first 5 days, and thereafter the ewes were offered 750 g day^{-1} of lupin grain. *Significantly different from control values ($P < 0.01$).

decrease was not significantly different from that in ewes that were receiving the straw diet alone.

Plasma gonadotrophins

There were no significant differences between the control and lupin-fed ewes for any of the LH measurements. The LH pulse frequency, the mean pulse interval, the mean pulse amplitude, the nadir and the mean concentrations were 5.1 ± 0.6 and 4.6 ± 0.6 pulses per 24 h, 335 ± 39 and 328 ± 44 min, 1.3 ± 0.1 and $1.4 \pm 0.2 \text{ ng ml}^{-1}$, 0.26 ± 0.05 and $0.24 \pm 0.05 \text{ ng ml}^{-1}$ and 0.48 ± 0.05 and $0.46 \pm 0.08 \text{ ng ml}^{-1}$ for the lupin-supplemented and control groups of ewes, respectively. There were no significant differences in mean FSH concentrations or significant changes with time (Fig. 2a).

Plasma prolactin

There was a significant ($P < 0.05$) increase in mean concentration of plasma prolactin in the ewes fed a lupin grain supplement

Fig. 2. The mean ($\pm$ SEM) (a) plasma FSH, (b) prolactin and (c) GH concentrations in ewes fed a basal diet of straw (○) or straw supplemented with lupin grain (●). Prolactin and FSH concentrations were determined every hour while GH concentrations were determined every 20 min. Bars representing the SEM are presented on only every fourth sample for GH. *Significantly different from control values ($P < 0.05$). The GH concentrations are significantly different at all times except samples 12 and 13 (at 5 h and 5 h 20 min).

(Fig. 2b). There were also significant effects of time ($P < 0.001$) and a treatment $\times$ time interaction ($P < 0.001$). The prolactin concentrations in the ewes fed straw alone began to increase from about 12 h after feeding (21:00 h) and remained high until the end of the sampling period. The plasma concentrations in

the lupin fed ewes began to increase about 5 h (13:00 h) after feeding, and this increased concentration was maintained throughout the sampling period. The concentrations in both treatment groups were similar from about 20 h (05:00 h) after feeding until the end of the blood sampling period.

Fig. 3. The mean ($\pm$ SEM) plasma insulin concentrations in ewes fed a basal diet of oat straw (□) or straw supplemented with lupin grain (■). The concentration of insulin was determined every hour between 1 h and 5 h, and between 20 h and 24 h after feeding.

Plasma growth hormone

Lupin supplementation had a significant effect ($P < 0.001$) on the mean concentration of plasma GH, and the concentrations were much lower than in ewes on the basal straw diet (Fig. 2c). There was a significant effect of time ($P < 0.01$) and a significant treatment $\times$ time interaction ($P < 0.01$). The release of GH in the straw-fed animals appears to have been synchronized and there were five apparent peaks of GH that occurred at intervals of 5–6 h. A similar pattern of release was not so evident in the lupin-supplemented group. In the lupin-supplemented ewes, there was a significant increase in concentrations about 4 h after feeding. This was also the only time that concentrations in the lupin-supplemented group reached concentrations observed in the straw-fed animals.

Plasma insulin

The insulin concentrations were significantly higher ($P < 0.02$) in the lupin-supplemented ewes (Fig. 3). The mean insulin concentrations for the two sampling periods were 2.7 ± 0.1 and 2.4 ± 0.1 ng ml⁻¹, respectively. For the ewes fed straw alone, the mean insulin concentrations over the same periods were 0.6 ± 0.1 and 0.4 ± 0.1 ng ml⁻¹, respectively.

Plasma IGF-I

The mean ($\pm$ SEM) concentrations of IGF-I in jugular venous plasma from the lupin supplemented ewes was 85.6 ± 5.1 ng ml⁻¹ and in the ewes not given the supplement, 84.7 ± 8.1 ng ml⁻¹. The difference was not significant.

Plasma amino acids

There were significant differences between the plasma concentration of 15 amino acids and urea of the control and

lupin-fed ewes (Table 2). The concentrations of nine of the amino acids were increased and six were decreased in the plasma of lupin-supplemented ewes.

Discussion

The ovulation rate was increased in lupin-fed ewes in the study reported here without significant alterations in the pattern of gonadotrophin secretion. However, the metabolic hormones, insulin, prolactin and GH were significantly altered by lupin feeding.

The mechanism by which lupin feeding results in an increase in ovulation rate is still unclear. Supplementation of ewes for 7 days with lupin grain did not affect the number of follicles > 2 mm in diameter at luteolysis but by 48 h after luteolysis there was a significantly greater number of follicles > 2 mm in diameter (Nottle *et al.*, 1985). This indicates that the increase in the number of ovulatory follicles is probably the result of decreased atresia in gonadotrophin-sensitive follicles.

The LH pulse pattern in ewes is unaffected by supplementation with lupin grain (Ritar and Adams, 1988; Radford *et al.*, 1980). This is confirmed by the present study and suggests that LH pulse frequency during the luteal phase of the oestrous cycle has no role in determining ovulation rate (Thomas *et al.*, 1984). While the present study found no change in FSH concentrations after lupin supplementation, it has been reported that small increases at about the time of luteolysis increase ovulation rate (McNatty *et al.*, 1985). The present data do not preclude a role for changes in FSH concentration during luteolysis mediating nutritional effects on ovulation rate.

There is an apparent circadian rhythm in circulating prolactin concentrations in both groups (Karsch *et al.*, 1989; Jackson and Jansen, 1991). Superimposed on this rhythm is a transient response in plasma prolactin between 4 h and 8 h after feeding lupin grain. Plasma prolactin rises to a peak several hours after

Table 2. The mean ($\pm$ SEM) plasma concentrations (nmol ml⁻¹) of amino acids, urea and ammonia (NH₃) in ewes fed straw or straw supplemented with lupin grain (lupin) on days 2–13 of the oestrous cycle. A blood sample was taken at 23 h after feeding on day 11 of the oestrous cycle

Amino acid, urea or NH ₃	Lupin		Straw		Significance of difference
Phosphoserine	4.8 $\pm$ 0.2		4.4 $\pm$ 0.4		NS
Taurine	32 $\pm$ 4		47 $\pm$ 8		a
Aspartic acid	1.1 $\pm$ 0.1		1.8 $\pm$ 0.2		a
Threonine	107 $\pm$ 8		37 $\pm$ 5		b
Serine	133 $\pm$ 9		136 $\pm$ 6		NS
Asparagine	56 $\pm$ 8		12 $\pm$ 6		b
Glutamic acid	38 $\pm$ 3		67 $\pm$ 6		b
Glutamine	609 $\pm$ 83		439 $\pm$ 22		NS
Alpha amino adipic acid	14 $\pm$ 1		14 $\pm$ 1		NS
Glycine	480 $\pm$ 20		733 $\pm$ 37		b
Alanine	132 $\pm$ 45		183 $\pm$ 13		a
Citrulline	180 $\pm$ 21		59 $\pm$ 3		b
Alpha amino butyric acid	26 $\pm$ 2		19 $\pm$ 1		a
Valine	171 $\pm$ 12		120 $\pm$ 4		b
Methionine	17 $\pm$ 1		10 $\pm$ 1		b
Isoleucine	59 $\pm$ 6		33 $\pm$ 1		b
Leucine	61 $\pm$ 7		49 $\pm$ 2		NS
Tyrosine	47 $\pm$ 5		13 $\pm$ 1		b
Phenylalanine	67 $\pm$ 7		61 $\pm$ 6		NS
Ornithine	90 $\pm$ 7		50 $\pm$ 3		b
Lysine	116 $\pm$ 9		93 $\pm$ 6		NS
Histidine	67 $\pm$ 3		62 $\pm$ 3		NS
1-Methyl-histidine	57 $\pm$ 8		60 $\pm$ 6		NS
3-Methyl-histidine	70 $\pm$ 8		129 $\pm$ 4		b
Arginine	124 $\pm$ 9		43 $\pm$ 2		b
Urea	9266 $\pm$ 379		795 $\pm$ 80		b
NH ₃	61 $\pm$ 5		49 $\pm$ 5		NS

a: significantly different ($P < 0.05$); b: ($P < 0.01$); NS: not significant.

feeding (McAtee and Trenkle, 1971), compared with rapid increases in stress responses (Neill, 1970; Lamming *et al.*, 1974). The increase in this experiment is more likely to be a metabolic rather than a stress response. The role, if any, that prolactin plays in follicular responses to nutrition remains unclear. Prolactin affects several ovarian steroidogenic pathways by influencing the availability of substrate used in the synthesis of steroids (Bartke *et al.*, 1987; Rajkumar *et al.*, 1985, 1988). It inhibits androgen synthesis by thecal cells and can suppress *in vivo* production of C19 steroids in the sheep ovary (McNeilly, 1984). The higher concentrations of prolactin in lupin-fed ewes may inhibit androgen synthesis, as has been reported for gilts given prolactin, in the late luteal phase of the oestrous cycle (Ciereszko and Duszka, 1993) and, through limiting oestradiol secretion, so favour the development of more than a single ovulatory follicle.

There were large differences in the concentrations of circulating GH between the groups. Since, in ruminants, plasma GH concentrations are high when the nutrient intake is low (Breier *et al.*, 1986; Hart *et al.*, 1978), the higher mean GH concentrations in the straw-fed animals were probably related to the

low nutrient intake of this group. Low nutrient intake and low body mass are also associated with high circulating GH concentrations and low ovulation rates (Kelly *et al.*, 1983). Since feeding the straw diet was associated with high GH concentrations but a lower ovulation rate, high circulating GH concentrations may inhibit the development of ovulatory follicles.

Many of the anabolic actions of GH are mediated through the hepatic and peripheral production of insulin-like growth factor I (IGF-I), which has important effects on ovarian cellular function. The peripheral concentrations of IGF-I in lupin-fed and straw-fed ewes were not different and this result concurs with those of Davis *et al.* (1990), who gave ewes daily injections of GH for 16–17 days and reported that plasma IGF-I concentrations were increased but the ovulation rate was not. Therefore, an increase in peripheral IGF-I does not appear to be the stimulus necessary for an increase in ovulation rate.

However, a differential effect of feeding on the local production of IGF-I in the ovary should not be discounted. When nutrient intake is limiting, GH concentrations are increased, but the ability of GH to stimulate IGF-I is uncoupled, so that GH no longer has anabolic effects on tissues (Muller and Locatelli, 1992). Therefore, the high peripheral concentrations of GH due to low nutrient intake may decrease ovarian concentrations of IGF-I, which could be detrimental to follicle development. The GH concentrations in well-fed ewes, although low, could stimulate follicle development through the anabolic actions of locally produced IGF-I. These observations and the low GH concentrations in lupin-supplemented ewes suggest that, if GH is involved in nutritionally induced increases in ovulation rate, high GH concentrations are inhibitory.

Nearly 60% of a dose of chromic oxide, an indigestible marker used in digestion studies (Faichney, 1972), passes through the duodenum of sheep in 4 h. The movement of nutrients into the abomasum could be related to the increased prolactin and GH concentrations observed 4–8 h after feeding.

The feeding of the lupin grain produced changes in the circulating plasma amino acids concentrations in the study reported here. The changes in the blood amino acid profile could have been derived from an increase in the protein escaping degradation in the rumen or from a significant increase in the amount of microbial protein entering the abomasum from the rumen. The amino acid composition of bacterial and protozoal protein remains relatively constant (Purser and Buechler, 1966; Bergen *et al.*, 1968). Microbial protein is deficient in certain amino acids such as methionine, lysine, histidine and arginine (Strom *et al.*, 1983). Therefore, any protein that supplies significant amounts of these limiting amino acids and which bypasses degradation in the rumen will have a major influence on the concentrations in the blood. Lupin feeding resulted in increased concentrations of the branched chain amino acids (422 nmol ml⁻¹ compared with 276 nmol ml⁻¹). The infusion of the branched chain amino acids, leucine, isoleucine and valine, for 5 days during the luteal phase increased ovulation rate in ewes (Downing, 1994). Therefore, higher plasma concentrations of the branched chain amino acids found following lupin feeding may play a role in the increased ovulation rate in the lupin-fed ewes.

Feeding lupin grain to ewes increases the digestible organic matter (Lindsay *et al.*, 1980) because about 35% of lupin protein escapes degradation in the rumen and passes into the small intestine (Hume, 1974). An increase in protein intake increases the metabolic rate (Egan, 1965) and, therefore, promotes an anabolic state and glucose uptake. The ovulation rate responses to feeding a high protein supplement are strongly related to the glucose entry rate into the cellular compartment from the extracellular compartment (Teleni *et al.*, 1989a,b). Plasma insulin concentrations were high in the lupin-fed ewes, as a result of increased availability of gluconeogenic amino acids. Therefore, it is highly probable that lupin-fed ewes have increased glucose availability and this may be the stimulus responsible for ovulation rate increases in response to high protein supplements. The increased ovulation rate in the lupin-fed ewes may also involve direct cellular actions of insulin (Poretsky and Kalin, 1987). Insulin has effects on follicular cell functions *in vivo* and *in vitro*.

There is no clear indication of how an increase in energy supply could influence follicular function but direct gonadal action of increased glucose availability and the anabolic actions of insulin may be important.

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