Actes Inst. Agron. Vet. (Maroc) 1997, Vol. 17 (3) : 133-141

Effect of increasing dietary energy and lasalocid treatment on gonadotrophin secretion in rams

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(Received 02 /12/1996 ; Accepted 06/03/1997)

فعل زيادة الطاقة الغذائية والمعالجة بالأزالوزيد على إفرازات الهرمونات منشطة المناسل عند الخرفان

إن التكملة الغذائية بحبوب الترمس يهيج إفراز الهرمونات منشطة المناسل. و لدراسة مدى تأثير هذا الفعل على التوالد بحصص غذائية مختلفة، تم تغذية الخرفان بحصة أساسية (1 كلغ علف، 10٪ حبوب الترمس، و 2,5٪ معدنية) أو حصة ذات طاقة عالية (50٪ علف برسيم، 27,8 ٪ علف مسحوق، 20٪ كلأ الشعير و 2,5٪ معدنية) بنسبة يومية 2,5٪ من الوزن الحقيقي للخروف لمدة 7 أيام. في آخر هذه المرحلة، نصف المجموعة الأولى تناولت 750غ/ رأس في اليوم بينما نصف المجموعة الثانية تناولت 45 ملغ لزالوسيد مع كل كلغ غذاء لمدة 10 أيام. أما باقي الخرفان فقد استمرت في تناول الحصة الغذائية المعطاة في البداية. تم أخذ عينات من الدم على رأس كل 20 دقيقة و على مدار 24 ساعة في اليوم :0 ، 7 و 17 بالنسبة لبداية العلاجات واستعملت في تقدير هرمون LH. وأخذت عينات من الدم عند كل ساعتين لتقدير كمية الحاثة منشطة الجريب (FSH) يوم 0 ، 7 و 17 و الأنسولين و الجلوكوز يومي 7 و 17. وأخذت عينات من سائل المعدة مأخود من الخرفان تحت حمية ذات طاقة مرتفعة في اليوم 7 و 17 واستعملت لتحليل الحوامض الدهنية المتبخرة. أظهرت النتائج أن الغذاء المتكون أساسا من حبوب الترمس أو أغذية غنية بالطاقة قادرة على تنشيط الهرمونات منشطة المناسل. هذه الأفعال هي بصفة عامة مشتركة مع تركيز بلازمي مرتفع للجلوكوز و الأنسولين.

الكلمات المفتاحية : الطاقة – لازالوسيد – الجلوكوز – الأنسولين – هرمونات منشطة المناسل – غنم

Effet de l'augmentation de l'alimentation calorique et du traitement au lasalocid sur la sécrétion des gonadotrophines chez les béliers

Chez le bélier adulte, la complémentation alimentaire par les grains de lupin (*Lupinus angustifolius*) stimule la secrétion des gonadotrophines. Pour tester la reproduction de cet effet par des rations alimentaires differentes, des béliers ont été soumis à une ration de base (1 kg de foin, 10% grain de lupin et 2,5% minéraux) ou une ration à niveau énergétique élevé (50% foin de luzerne, 27.5% foin broyé, 20% orge fourrager et 2,5% minéraux) au taux quotidien de 2.5% du poids vif pendant 7 jours. À la fin de cette période, la moitié du premier groupe a reçu 750 g/tête . jour alors que la moitié du deuxième groupe a reçu une dose orale de l'ionophore lasalocid (45 mg/kg d'aliment) pendant 10 jours. Le reste des béliers a continué à reçevoir leur ration alimentaire de départ. Des prises de sang ont été faites toutes les 20 min pendant 24 h à 0, 7 et 17 jours par rapport au début des traitements et ont été utilisées pour le dosage de l'hormone luté inisante. Des prises de sang toutes les 2 heures ont servi au dosage de l'hormone folliculo-stimulante (Jours 0, 7 et17), de l'insuline et du glucose (Jours 7 et 17). Des échantillons de liquide ruminal, pris sur les béliers sous régime riche en énergie à 7 et 17 jours, ont été utilisés pour l'analyse des acides gras volatiles. Les résultats montrent que l'alimentation à base de lupin ou d'autres aliments riches en énergie est suceptible de stimuler les secrétion de la LH et de la FSH. Ces effets sont généralement associés avec des concentrations plasmatiques élevées de glucose et d'insuline.

Mot clés: Alimentation calorique- Lasalocid - AGV- Glucose - Insuline - Gonadotrophines - Ovin

Effect of increasing dietary energy and lasalocid treatment on gonadotrophin secretion in rams

In the mature ram, supplementary feeding, for example with lupin grain, stimulates gonadotrophin secretion. To test whether this effect is reproducible with alternative diets not containing lupin grain, rams were fed either a basal diet (1 kg of oat hay containing 10% lupin grain and 2.5% minerals) or a high energy diet (50% lucerne chaff, 27.5% hammer-milled oat hay, 20% rolled barley and 2.5% minerals) at the daily rate of 2.5% of live weight for 7 days. After this period, half of the first group received 750 g/head daily whereas half of the second group received a daily drench of an ionophore, lasalocid (45 mg/kg of food) for 10 days. The other rams remained on their respective diets. Blood was sampled every 20 min for 24 h on days 0, 7 and 17 relative to the start of treatments for the study of plasma LH pulse profiles. Plasma samples taken every other hour were assayed for FSH (days 0, 7 and 17), insulin and glucose (days 7 and 17). Rumen fluid samples taken, from rams fed the high energy diet, on days 7 and 17 were analysed for volatile fatty acid content. It is concluded that feeding either lupins grain or a high energy diet stimulates LH and FSH secretion. These effects are associated with increased plasma concentrations of glucose and insulin

Key words: Dietary energy - Lasalocid - VFA - Glucose - Insulin - Gonadotrophin - Sheep

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INTRODUCTION

The ability of lupin feeding to stimulate gonadotrophin secretion and testicular growth when given as a supplement to rams (Boukhliq, 1993) has highlighted the need to identify other food substitutes that can be used for the same purpose in areas where the use of lupin grain in animal husbandry is not a common practice. These alternatives could include legumes and grains to substitute for the lupin grain or drugs that interfere with the pathway(s) involved in determining the response to lupin feeding. Available data indicates that lupin feeding is associated with increased production of glucose, but glucose treatment does not reproduce the *so called* 'lupin effect' on gonadotrophin secretion (Boukhliq, 1993). Other metabolites associated with glucose metabolism such as volatile fatty acids in general and propionate in particular may be involved in the reproductive response to supplementary feeding.

The ionophorous antibiotic lasalocid (Bovatec™, Hofman La Roche, Inc., Nutley, NJ) has been found to increase weight gain of ewes, and to improve lamb survival (Shetaewi & Ross, 1977) and hasten the onset of puberty in heifers (Mosely *et al.*, 1977) and bulls (Rutter *et al.*, 1991). The mechanisms controlling these effects are unclear but have been suggested to involve a change in rumen fermentation patterns. Specifically, an effect on volatile fatty acid (VFA) production resulting in an increased propionate to acetate ratio, thus an increase in the primary gluconeogenic precursor (Richardson *et al.*, 1976). Further studies with ionophore-fed prepubertal heifers and bulls have demonstrated an enhanced LH and testosterone responses to injections of either GnRH or oestradiol-17ß (Randel & Rhodes III, 1980; Randel *et al.*, 1982; Rutter *et al.*, 1991) and an increased ovulation rate following FSH treatment (Bushmich *et al.*, 1980). Although these and other beneficial effects have been attributed to the ability of the ionophore to alter volatile fatty acids proportions and rumen fermentation, little is known about changes in blood chemistry and hormone profiles associated with feeding lasalocid in the male sheep.

This experiment investigated the relative importance of a high energy diet that did not contain lupin grain as well as precursors of glucose on gonadotrophin secretion in mature rams. We tested the following hypotheses:

- that feeding a high energy diet will stimulate the secretion of gonadotrophins in a similar way to the lupin grain supplement, and
- that feeding lasalocid to rams on a high energy diet will increase the molar proportions of propionate to acetate and butyrate, thereby increasing the peripheral concentrations of insulin. This would lead to a stimulation of gonadotrophin secretion (which would ultimately result in greater testicular growth).

MATERIALS AND METHODS

Twenty four mature Merino rams were housed in individual pens in the animal house at the University of Western Australia (latitude: 34°S) for an acclimatization period of 3 weeks. Half of the rams were used as controls and were fed a diet designed to maintain live weight (1 kg oat hay with 10% lupins daily and 2.5% minerals (Siromin™, Narrogin Mineral Stockmix, Narrogin, Western Australia)) for 7 days and then the same diet with (Lupin group) or without (Maintenance group) a lupins supplement (750 g/day) for 10 days. The remaining animals were fed a high energy diet for a week (High energy groups) and then half of them, chosen randomly, were given a lasalocid drench before feeding each day for 10 days (Fig. 1). The high energy diet was used at the daily rate of 2.5% of live weight and consisted of 50% lucerne chaff, 27.5% hammer-milled oat hay, 20% rolled barley plus 2.5% minerals. The estimated metabolizable energy and crude protein (CP) content of the different diets were 6.28 MJ/d and 46 g CP/d for the maintenance group, 17.35 MJ/d and 274.21 g CP/d for the lupin-supplemented group and 23.06 ± 0.59 MJ/d and 191.69 ± 4.94 g CP/d for the high energy groups respectively. Lasalocid (Bovatec™, Colborn Dawes Aust. Pty Ltd., N.S.W.), a biologically active ionophore produced by *Streptomyces lasaliensis* was used at the rate of 45 mg/kg of feed.

Blood was sampled from all rams every 20 min for 24 hours on days 0, 7 and 17 of the treatment period (Figure 1). All plasma samples were assayed for LH while 2-hourly subsamples were pooled together before being assayed for FSH. Four-hourly samples were assayed for glucose and insulin as described earlier (Boukhliq *et al.*, 1996). Rumen fluid was sampled from the high energy fed rams, 5 hours after feeding on days 7 and 17. Rumen fluid samples were taken using a rumen sampler. Nine millilitres of strained rumen fluid were added to 1 ml of 1M sodium hydroxide and then centrifuged at 3000 rpm for 10 min. The supernatant was decanted and frozen until analysis for volatile fatty acids (VFA) by gas liquid chromatography (Harman, 1991).

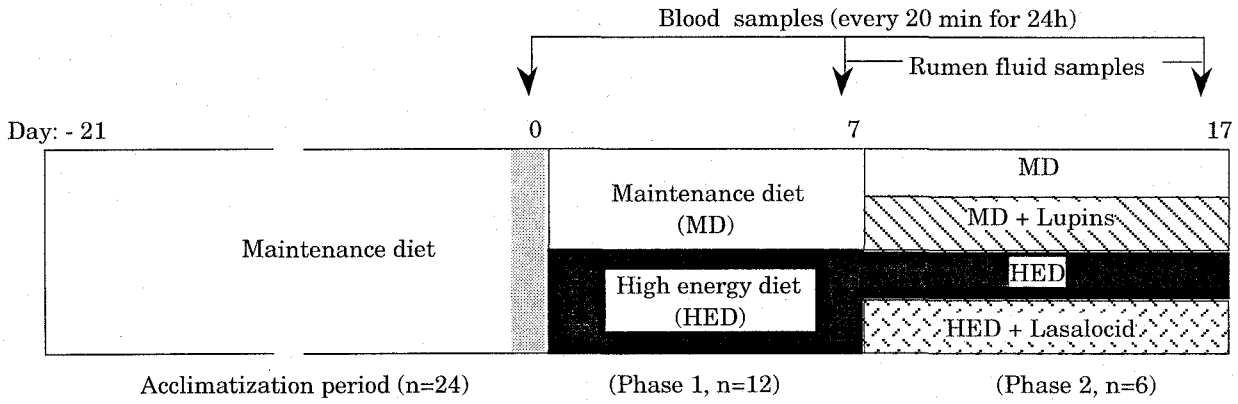

Figure 1. Design of the experiment showing treatments groups and sampling schedule

For determination of glucose concentrations, plasma samples were deproteinized according to the method of Arthur *et al.* (1989). The concentration of glucose in deproteinized samples was measured by the glucose oxidase method of Bergmeyer & Bern't (1974) as modified by Holmes (1990). The limit of detection was 0.1mM. Within-assay variation was estimated in each assay using at least 2 replicates of 2 pooled plasma samples containing 3.66 (1.4 ± 0.06%) or 6.14 mM (1.4 ± 0.09%). The between-assay coefficients of variation were 7.6% and 8.2% respectively.

Insulin was measured using the double antibody radioimmunoassay developped by P. Wynn (CSIRO, Prospect, New South Wales, Australia) based on the assay previously described by Bassett (1974). In brief, A 100 µl plasma sample, or standard, was preincubated with guinea pig antiserum to porcine insulin (100 µl, final dilution 1:125 000 in phosphate buffered saline (0.25% BSA) and ^{125}I-porcine insulin (100 µl at approximately 10,000 cpm) for 24 h at 20°C. After incubation, bound hormone was precipitated using 100 µl of donkey antiserum to guinea-pig gamma globulin and 100 µl of normal guinea pig serum. Tubes were centrifuged at 3000 rpm for 30 min at 4°C, supernatant was aspirated and the precipitate (bound hormone) was counted using a gamma counter. The antiserum (GP2, 21/7/80) was kindly donated by Dr Peter Wynn. It was prepared in guinea pig using bovine insulin (BI 4499, Eli Lilly). Cross-reactions were 100% with ovine insulin (Sigma I9254 lot 57800667), 100% with bovine insulin (NOVO, lot 016666), 56% with porcine insulin (NOVO, lot 521166), 63% with equine insulin (NOVO lot AC1131262A), 30% with bovine pro-insulin (Dairy Research Unit-D Leenanuruska), and less than 1% with somatostatin (Ref No A14907, Peptech Technology), beef glucagon (NOVO, lot A66) or ovine GH (NIH NIDDK-064-I4). Porcine insulin with a potency of 26.8 units/mg (Lilly Laboratories, Indianapolis, Indiana) was used as reference, and as a tracer after it was labelled with iodine-125 by the chloramine-T method (Greenwood *et al.*, 1963). Cross reactions were provided by Dr P. Wynn (CSIRO, Prospect, New South Wales, Australia). The limit of detection was 1.8 ± 0.2 µU/ml plasma. The non specific binding was 2.4 ± 0.35%. Included in each assay were six replicates of three pooled plasma samples containing 10.19, 25 and 111.9 µU/ml. They were used to estimate the coefficients of variation within assays (8.25 ± 0.7, 6.09 ± 0.4 and 14.5 ± 0.7%) and between assays (10.05, 11.8 and 25.4%).

Plasma concentrations of LH were measured in all samples. The assay technique was similar to that reported elsewhere (Martin *et al.*, 1980). The samples were assayed as duplicate 200 µl aliquots. The limit of detection was 0.31 ± 0.1 ng/ml. Within-assay variation (mean ± SEM) was estimated in each assay using at least 5 replicates of three pooled plasma samples containing 0.32 (16.4 ± 2.5%), 2.2 (12.1 ± 1.9%) or 5.23 ng/ml (9.4 ± 3.2 %). The between-assay coefficients of variation were 15.3%, 13.8% and 7.0%, respectively, and the effect that this would have had on the detection of LH pulses was avoided by assaying all samples from an animal in the same run. The two pooled samples with low concentrations were used to improve the reliability of pulse detection around the baseline of each LH profile. The serial samples were analysed with a modified version of the 'Pulsar' algorithm developed by Merriam and Wachter (1982) and modified for the Apple computer ('Munro', Zaristow Software, West Morham, Haddington, East Lothian EH41 4PD, U.K.), as described by Martin *et al.* (1986a). The G Parameters (the number of standard deviations by which a peak must exceed the baseline in order to be accepted) were 3.98, 2.8, 1.68, 1.34,

and 0.93 for G1-G5, these being the requirements for pulses composed of one to five samples that exceed the baseline, respectively. The Baxter parameters describing the parabolic relationship between the concentration of hormone in a sample and the standard deviation (assay variation) about that concentration were 0.34862 (b1, the y intercept), 0.02548 (b2, the x coefficient) and 0.01161 (b3, the x^2 coefficient). The pulse frequency, the mean pulse amplitude (the difference between pulse peak and preceding nadir) and the mean level of LH were calculated for each profile.

Plasma concentrations of FSH were assayed in 2-hourly samples from the intensive sampling, using a previously described assay (Atkinson & Adams, 1988). The samples were assayed in duplicate 200µl aliquots. The limit of detection was 0.27 ng/ml. Within-assay variation was estimated in each assay using at least 5 replicates of three pooled plasma samples containing 1.3 (11.5 ± 1.9%), 3.2 (10.2 ± 1.1%) or 7.34 ng/ml (10.9 ± 0.6%). The between-assay coefficients of variation were 34.5%, 14.6% and 11.67% respectively.

• Statistical analysis

LH profiles were analysed for pulses using "Munro" (Elsevier-BIOSOFT, Cambridge, UK). Data for LH parameters, FSH, VFA, glucose and insulin were compared using repeated measures analysis of variance with values of day 0 as covariates.

RESULTS

• Volatile fatty acids

The molar proportions of different volatile fatty acids in the rumen fluid in the rams fed the high energy diet are presented in table 1. As expected in the control rams (high energy diet only), there were no significant differences between days 7 and 17 in any of the VFA measured. In the lasalocid-treated rams, a comparison of the values before and after treatment showed that the molar proportion of propionate increased by 13.9% ($p < 0.05$), while the proportions of acetate and butyrate decreased by 8% and 16% respectively ($p < 0.05$). There was also a significant increase of 37.6 % of the propionic/acetic ratio ($p < 0.05$). Comparisons of the values recorded on day 17 for the high energy- and lasalocid-fed rams showed a 49.7 % increase in the molar proportion of propionate ($p < 0.05$), a 8.4 % and 30.0 % decrease in acetate and butyrate respectively ($p < 0.05$) and a 64.4% increase in the propionic/acetic ratio ($p < 0.05$) as a result of lasalocid treatment. No significant differences were evident for isovalerate or valerate in any of the comparisons made.

• Glucose and insulin concentrations

Repeated measures analysis of variance revealed no significant differences between the maintenance and high energy groups for either glucose or insulin concentrations on day 7 of the treatment period (Figure 2). However, the decline in insulin concentrations after feeding differed between treatment groups. When a quadratic line was fitted, the differences in slope and curvature of the line at 2 hours after feeding were significant ($p < 0.05$). Insulin concentrations in the rams fed to maintenance declined exponentially over time, while in the high energy group the decline was linear.

On day 17 of the treatment period, repeated measures analysis of variance revealed that there were significant effects of treatment ($p < 0.05$), time

Table 1. Molar proportions of volatile fatty acids in rumen fluid of rams fed a high energy diet and treated or not with lasalocid at the daily rate of 45 mg/kg. Means in the same row with different superscripts differ ($p < 0.05$), *Means in the same row differ from control group after treatment ($p < 0.05$)

Fatty acids	Control (High energy)		High energy + lasalocid	
	Day 7	Day 17	Day 7	Day 17
Acetic (C_2)	70.71 ± 0.94^a	68.89 ± 0.92^a	68.58 ± 0.63^a	63.05 ± 1.10^{b*}
Propionic (C_3)	20.04 ± 0.87^a	19.49 ± 0.94^a	25.6 ± 0.91^a	29.18 ± 1.25^{b*}
Butyric	8.16 ± 0.27^a	9.41 ± 0.46^a	7.84 ± 0.58^a	6.57 ± 0.59^{a*}
Isovaleric	0.17 ± 0.02^a	0.31 ± 0.12^a	0.48 ± 0.23^a	0.38 ± 0.16^a
Valeric	0.73 ± 0.04^a	0.46 ± 0.05^a	0.64 ± 0.09^a	0.47 ± 0.02^a

($p < 0.01$) and treatment x time interaction ($p < 0.01$) on glucose and insulin concentrations. When compared to the maintenance diet, both the lupin grain supplement and the high energy diet significantly increased plasma concentrations of glucose and insulin. Lasalocid treatment, however, did not result in any additional increase in plasma levels of glucose or insulin despite the significant increase in the molar ratio of propionate to acetate. Moreover, plasma glucose concentrations tended to be lower in the lasalocid-fed rams compared to the high energy group but this decrease was not significant (Figure 3). The lower concentrations of glucose observed in lasalocid-treated rams was

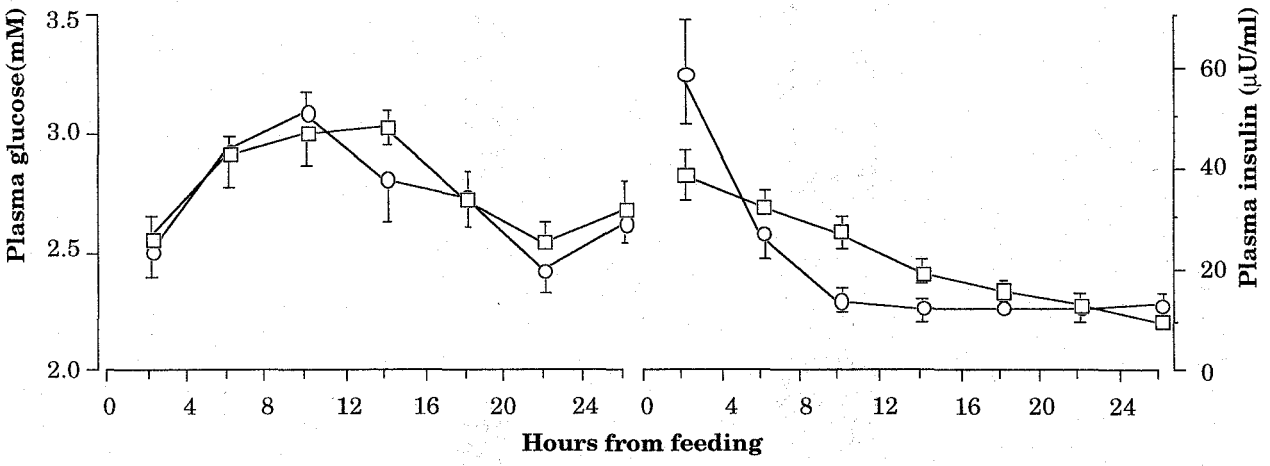

Figure 2. Plasma concentrations of glucose and insulin on day 7 in rams fed a maintenance diet (o–o) or a high energy diet (□–□)

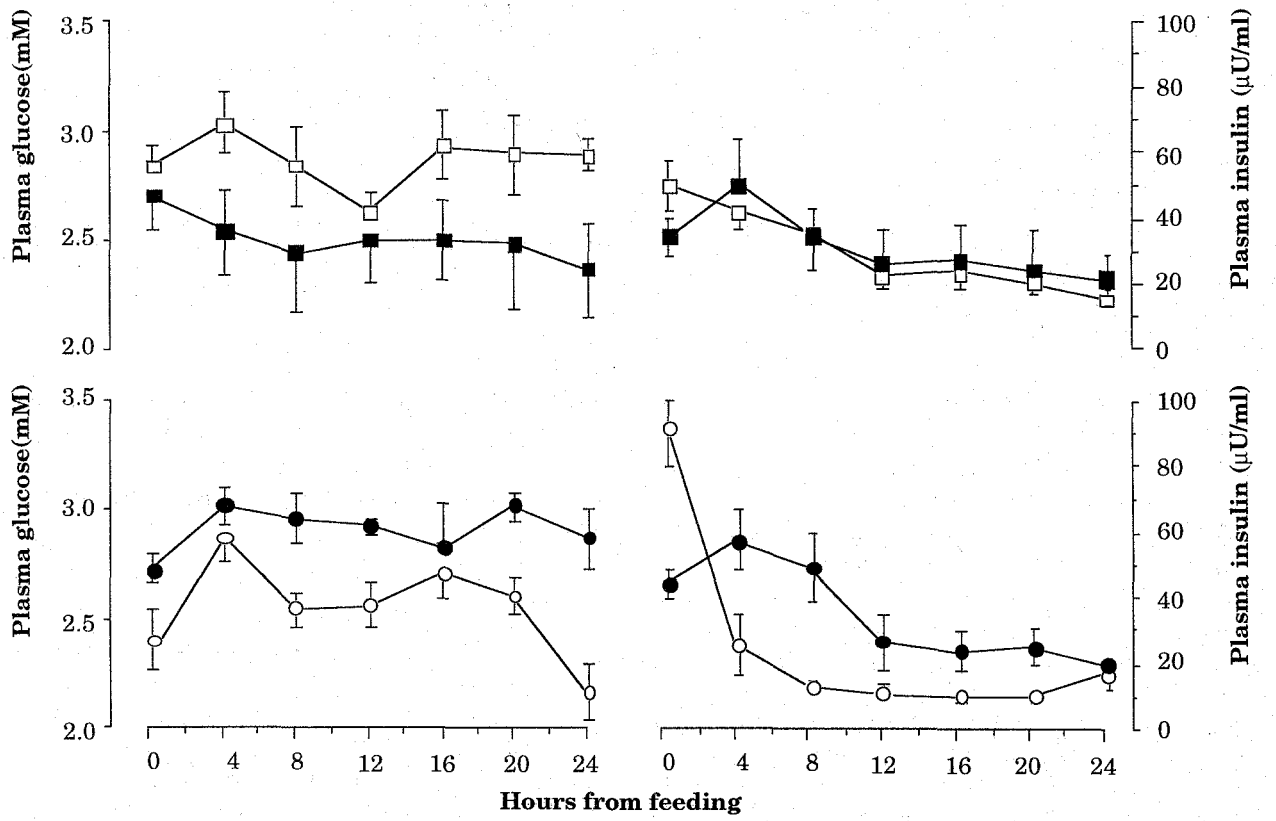

Figure 3. Plasma concentrations of glucose and insulin on day 17 in rams fed a high energy diet with (■–■) or without (□–□) a daily lasalocid drench or a maintenance diet with (●–●) or without (o–o) a lupin grain supplement

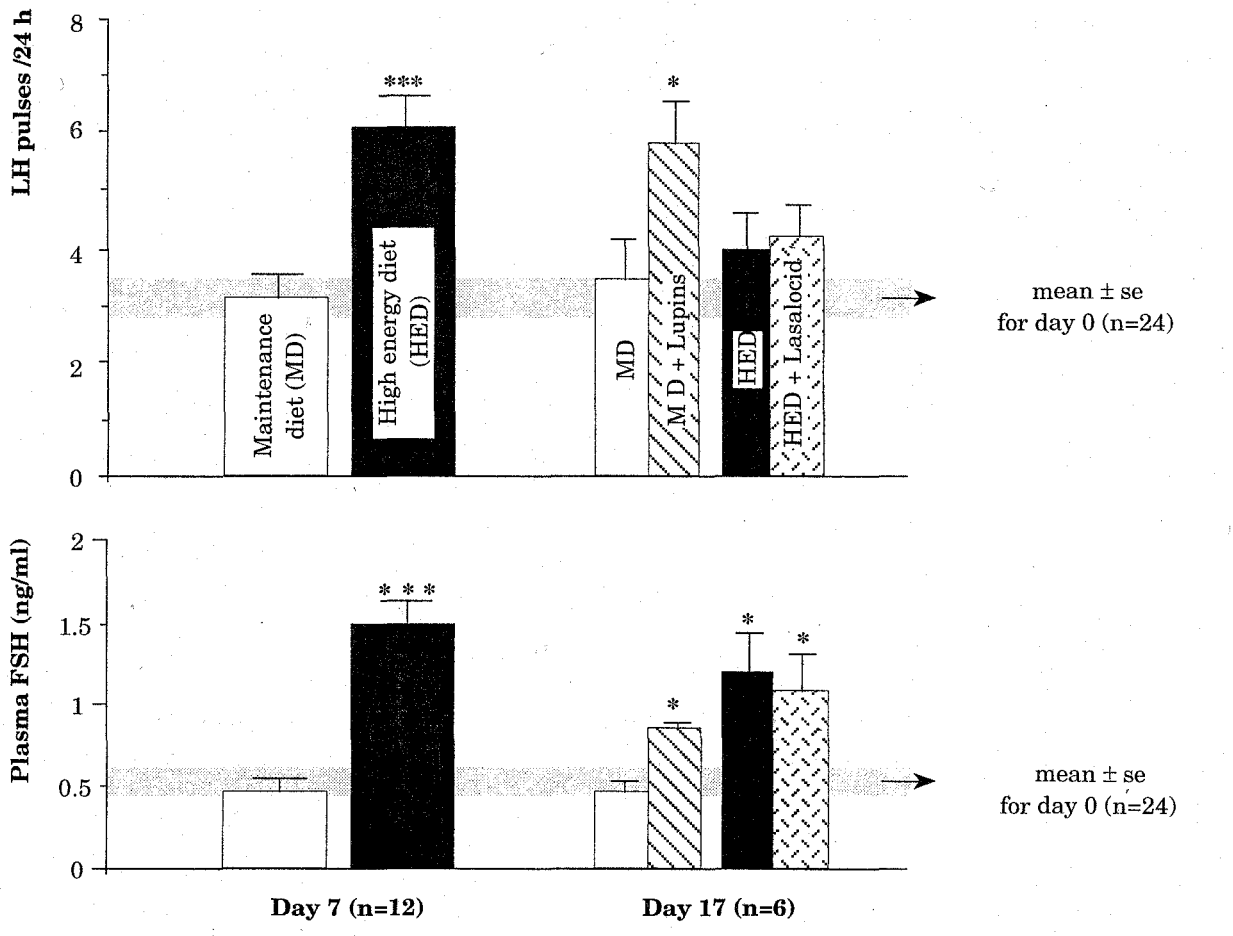

Figure 4. LH pulse frequency and plasma FSH concentrations in rams fed a maintenance diet (□) and supplemented with lupin grain (▨), or fed a high energy diet (■) and supplemented with lasalocid (▩)

caused by one ram in this group having very low plasma glucose concentrations. However, removing this ram from the analysis did not result in any statistical differences.

• LH and FSH secretion

Data for LH and FSH are presented in figure 4. Using pre-treatment values (day 0) as covariates, feeding the high energy diet for 7 days significantly increased LH pulse frequency and pulse amplitude ($p < 0.001$). However, this difference disappeared by day 17 even though the animals were fed the same diet between days 7 and 17. Neither LH pulse frequency, pulse amplitude or mean concentration were different between the rams fed the high energy diet and those fed to maintenance on day 17. There was a significant increase in plasma FSH concentrations when rams were fed a high energy diet and this effect was highly significant on day 7 (300% increase; $p < 0.001$) and on day 17 (100% increase; $p < 0.05$) of the treatment period. The lasalocid treatment did not have any additional effect to the high energy diet as there were no differences between the high energy-fed rams and lasalocid-treated animals (Figure 4).

Feeding lupins for 10 days enhanced LH and FSH secretion ($p < 0.05$). This was illustrated by a high LH pulse frequency and FSH concentration in the plasma of lupin-fed animals as opposed to rams fed to maintenance (Figure 4).

DISCUSSION

In this experiment we tested the hypotheses that: (i) increased gonadotrophin secretion in rams given a protein and energy supplement is due to increased intake of energy and protein and that it is the combined effect of energy intake and the availability of substrates to synthesize glucose which initiate the changes rather than the direct action of nutrients *per se*; and that (ii) providing more propionate through an ionophore modifying the pattern of

fermentation would potentiate the effect of the high energy and protein diet. The first hypothesis was accepted and the second was only partially supported as lasalocid treatment significantly increased the ratio propionate to acetate, but this was not translated into changes in blood levels of glucose, insulin or gonadotrophins.

Supplementation with lupin grain or feeding a high energy diet independently of lasalocid treatment was successful in rapidly stimulating the secretion of LH and FSH. This suggests that increases in nutritional status, whether supplied in the form of lupin grain or other high energy diets, stimulate gonadotrophin secretion and this would be expected to stimulate testicular growth should the treatment be maintained over a long period (e.g., > 1 month).

In this experiment, rams were exposed to the high energy diet for 17 days or fed a lupin supplement for 10 days. Both treatments stimulated the secretion of LH and FSH after a short exposure to the treatment. These results agree with previous studies (Ritar *et al.*, 1984; Martin *et al.*, 1989). However, on day 17, FSH concentrations were still elevated in the high energy group while LH pulse frequency decreased to concentrations not statistically different from those observed in the rams fed to maintenance. The early stimulation and the subsequent decrease of LH secretion has been observed in lupin-fed animals as well and is not easily understood. Perhaps LH only plays a permissive role in the control of testicular growth while FSH is responsible for subsequent support of testicular growth and spermatogenesis. Alternatively, the divergent response of LH and FSH might be that the control of FSH is independent of pulsatile LH secretion, even though they are controlled by GnRH from the hypothalamus. Possibly, the secretion of FSH is only stimulated at the pituitary level and not at the hypothalamus, while LH is stimulated at the hypothalamic level only.

Feeding a high energy diet or a lupin grain supplement significantly increased glucose concentrations in blood plasma above levels observed in the rams fed to maintenance. This increase in glucose availability was maintained over the 24 hour period measured on days 7 and 17. In the maintenance group where glucose was limiting, there was an exponential decline over time in insulin concentrations after feeding, whereas the decline for the high energy and lupin group was gradual and linear. Therefore, the sensitivity of the post prandial rise in insulin seems to depend on the plane of nutrition.

Whether changes in LH and FSH on a high plane of nutrition are mediated by changes in glucose and insulin is yet to be clarified. In an earlier experiment, we have shown that infusion of glucose does not increase the secretion of either LH or FSH and it was concluded that stimulation of the hypothalamo-pituitary axis does not involve the energy component glucose (Boukhliq *et al.*, 1996). It might be that propionate or other VFA act on the hypothalamo-pituitary axis directly if at all. However, this study provides more evidence that glucose is not an important nutrient acting at the hypothalamo-pituitary axis to stimulate gonadotrophin secretion (and consequently testicular growth).

The changes in fermentation patterns of rams fed lasalocid in addition to a high energy diet agree with previous findings (Richardson *et al.*, 1976; Neuendroff *et al.*, 1985; Aitchison *et al.*, 1989). These changes in the pattern of VFA fermentation, with large increases in the molar proportions of propionate, would suggest that more energy was available to the animal from VFA, with less demand for amino acids for use in gluconeogenesis. It is therefore surprising that there were no significant increases in blood concentrations of glucose and insulin in lasalocid-fed rams. Probably the amount of increase in propionate in the rumen, although reaching statistical significance, may not have been enough to be converted to glucose by the liver and consequently increase peripheral plasma glucose. Alternatively, glucose utilization may have increased leading to unchanged peripheral glucose levels. The efficiency of conversion of propionate into circulating glucose may be less than in the rumen and could not be confirmed as volatile fatty acids in blood plasma were not measured. Using dilution technique in a study with sheep, Steel and Leng (1973) found that only 40 to 60% of the propionate carbon produced in the rumen is converted to glucose. The metabolic fate of the remainder of the carbon from propionate is unknown. Further investigations, however, are warranted to determine how a shift in rumen VFA production will affect net hepatic uptake and peripheral levels of glucose.

The absence of a change in gonadotrophin secretion in lasalocid fed rams and the failure of this treatment to maintain the increased LH pulse frequency induced by the previous exposure to the high energy diet is in agreement with results of Kirkwood et al.

(1991) who reported no effect of monensin, a lasalocid equivalent, on GnRH stimulated gonadotrophin release in gilts. In contrast, Rutter *et al.* (1991) reported that lasalocid-fed prepubertal bulls released more LH and testosterone in response to GnRH than did control bulls. In the same study, lasalocid fed bulls were only 30 days younger than the control bulls at puberty. Negative effects of ionophores have also been reported. For example, when administered intravenously to steers at a high dose (40 mg), monensin but not lasalocid caused a depression of LH secretion (Armstrong & Spears, 1988). This effect was associated with alterations of extracellular concentrations of Mg, Ca and K which may have altered GnRH release or the affinity of GnRH for its receptor. In these studies and in many others (Randel & Rhodes III, 1980; Randel *et al.*, 1982; Neuendroff *et al.*, 1985), the response of lasalocid-fed animals was evaluated by the release of LH and/or testosterone to a single or a multiple challenge of GnRH, which indicates a change in pituitary stores rather than secretion. We were unable to find in the literature data on pulsatile LH secretion in ionophore-treated animals. Convincing evidence, however, suggest an effect of ionophores on metabolism independent of alterations in ruminal microbial metabolism. Thus, it appears that there is considerable uncertainty as to the mechanism by which ionophores influence reproductive efficiency. The mode of action of lasalocid on pituitary and testicular function remains uncertain and this study suggest that the brain is not responsive to lasalocid treatment.

CONCLUSION

Feeding either a high energy diet or a lupin grain supplement induces a time-dependent stimulation of gonadotrophin secretion in parallel with physiological changes in blood concentrations of glucose and insulin. Dietary lasalocid at the rate of 45 mg/kg of feed alters rumen fermentation by increasing production of propionate at the expense of acetate. Although this change is high and possibly of biological value, it does not significantly alter serum glucose and insulin nor does it affect gonadotrophin secretion in rams. Therefore results indicate that lasalocid feeding has no effect on the reproductive system in the ram and that no changes in hypothalamo-pituitary function occur in lasalocid fed rams. Further studies on the relationship between nutrition/metabolism, specific nutrients and reproductive physiology and efficiency are needed.

ACKNOWLEDGEMENTS

The work described in this paper would not have been feasible without the technical support and expertise of many people, particularly Sonya Butt, Norman Adams and Margaret Blackberry. We are grateful to Dr David Pethick (Murdoch University, Western Australia) for his help with VFA assays . For the reagents for the FSH assay, we are grateful to the National Institute of Diabetes, Digestive and Kidney Disease, the Center for Population Research of the National Institute of Child Health, the Agricultural Research Service of the U.S. Department of Agriculture, and the University of Maryland School of Medicine. The reagents for the insulin assay were kindly provided by Dr Peter Wynn (Department of Animal Science, Sydney University, NSW, Australia). This work was funded by the Australian Research Council and the CSIRO Division of Animal Production.

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