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Nutrition and reproduction in the ram in a Mediterranean environment

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SUMMARY - The stimulation of gonadotrophin secretion and testicular growth in lupin grain-supplemented rams is presented as a working model. This effect is characterized by three types of responses, the first of which is the stimulation of pulsatile secretion of LH. This response is expressed as a 2 to 3 fold increase in pulse frequency and is obtained after a period as short as 2-3 days, but thereafter it declines and becomes undetectable after 3-4 weeks. The second response is that of FSH which usually starts to be detectable 1 week to 10 days after supplementation has started and is maintained for several weeks. The third response, an increase in testicular size, is rarely detectable before 2 weeks but continues for at least 5 weeks. Nutritional cues seem to control testicular growth through changing the secretion of gonadotrophins. These changes do not fully explain the effects of supplementary feeding on reproduction in the ram. It is thus suggested that gonadotrophin-dependent mechanisms play a permissive role for testicular growth, but gonadotrophin-independent mechanisms are also involved in transducing the effects of nutrition on reproduction in the ram.

Key words: Nutrition, reproduction, Mediterranean environment, ram, review.

RESUME - *"Nutrition et reproduction chez le bélier dans un environnement méditerranéen". La stimulation de la sécrétion de gonadotrophine et la croissance testiculaire chez des béliers recevant un supplément grain-lupin, est présentée comme modèle de travail. Cet effet se caractérise par trois types de réponses, la première étant la stimulation de la sécrétion pulsatile de LH. Cette réponse est exprimée comme une augmentation par 2 ou 3 de la fréquence pulsatile et elle est obtenue après une période de seulement 2-3 jours, mais ensuite elle baisse et devient indétectable après 3-4 semaines. La deuxième réponse est celle de la FSH qui commence habituellement à être détectable 1 semaine à 10 jours après que la supplémentation ait commencé, et se maintient pendant plusieurs semaines. La troisième réponse, une augmentation de la taille testiculaire, est rarement détectable avant 2 semaines mais continue pendant au moins 5 semaines. Des signaux nutritionnels semblent contrôler la croissance testiculaire au travers de modifications de la sécrétion de gonadotrophines. Ces changements n'expliquent pas entièrement les effets de la supplémentation sur la reproduction chez le bélier. On suggère donc que des mécanismes dépendants de la gonadotrophine jouent un rôle permissif pour la croissance testiculaire, mais des mécanismes indépendants de la gonadotrophine sont également impliqués dans la transduction des effets de la nutrition sur la reproduction du bélier.*

Mots-clés : *Nutrition, reproduction, environnement méditerranéen, bélier, révision.*

Introduction

In the Mediterranean regions of the world, the nutritional demands for reproduction in the grazing animals must be met from pasture and, within limits, the quantity and the quality of the pasture are determined by climatic and rainfall patterns. For successful management of farm animals under these conditions, existing pastures have to be managed efficiently and supplementary feeding has to be targeted to achieve the optimum level of reproduction. This requires a clear understanding of both the nutrient requirement for reproduction and the mechanisms that partition nutrients between the various competing physiological systems. This paper outlines the relationship between nutrition, especially the case of supplementary feeding, and testicular growth using data derived from Merino sheep in a Mediterranean type environment (south Western Australia).

Nutrition as a seasonal cue

In a Mediterranean environment, the peak of pasture growth is in winter and spring, the pasture dries off over summer and very little is left by autumn. Under natural grazing conditions, nutrition can have a more important influence on testicular growth than photoperiod. Scrotal circumference shows a clear seasonal pattern, with a relatively rapid increase over winter-spring followed by regression during autumn, so that the minimum size is reached in winter. Testicular size as well as peripheral concentrations of follicle-stimulating hormone (FSH) tend to parallel the seasonal changes in live weight, and the peaks and nadirs of the cycle are observed at very different times from those that would be expected with photoperiodic control. Thus, regression and recrudescence of testicular size as well as the pattern of FSH secretion in Merino rams are closely related to live weight rather than to photoperiod.

When Merino rams were fed to maintenance, they showed a pattern of increasing scrotal circumference, starting in late spring after the spring equinox and reaching a maximum in autumn-early winter, then decreasing in mid-late winter after the winter equinox (Murray *et al.*, 1991). When they were supplemented with lupins for 7 weeks at three times of the year (winter, spring-summer and autumn), scrotal circumference responded to supplementary feeding in all 3 seasons and the magnitude of this response was higher in spring than in autumn and winter (Murray *et al.*, 1991). These results suggest that during the increased daylengths in spring, nutritional stimuli can override the inhibitory effect of photoperiod, resulting in testicular growth.

Nutrition and spermatogenesis

In Merino rams, spermatozoa are produced by the testes at a relatively constant rate of about 20×10^6 spermatozoa/g testis per day (Knight, 1977). There is also an established relationship between ram live weight and testicular size (Knight, 1977; Braun *et al.,* 1980), with big rams usually having bigger testes. For a particular breed, maximum testicular size is presumably limited by the genetic potential of each ram, with previous nutrition influencing testicular growth within the constraints of that potential. In general, testicular size determines the capacity to produce sperm (Oldham *et al.,* 1978). An interesting point from a practical point of view is that the volume of testes of rams usually falls during joining probably because of the reduction in food intake. However, the rate at which rams loose testicular volume is not influenced by the original volume at the start of joining or the ratio of rams to ewes (Lindsay *et al.,* 1976). This means that rams with superior daily sperm production apparently maintain that superiority throughout joining. Spermatogenic activity, sperm content of the epididymis, and other variables were also lower in underfed than in *ad libitum* fed rams. Improvement of testicular efficiency by feeding was also reported by Oldham *et al.* (1978) who showed that rams maintained on a high plane of nutrition produced more sperm than those raised under deficient nutrition.

Energy *vs* protein

In the Mediterranean environment of Western Australia, lupin seed is the most common legume fed as a supplement. It can be fed out *ad libitum* without an introductory period and supplies high levels of both energy and protein (16.4 MJ/kg DM; 337.5 g CP/kg DM) (Hove, 1974). The observation that feeding lupins for few days increases ovulation rate in ewes (Stewart and Oldham, 1986) led to the suggestion that short term changes in nutritional status would also affect testicular growth in rams, a hypothesis supported by the study of Oldham *et al.* (1978). Since then there has been interest in identifying the mechanisms for such responses and the metabolic and endocrine pathways involved. Broadly, two schools of thought exist, namely that the effect is due to the increased protein/nitrogen intake or that it is due to an increased metabolizable energy. Up to 800 g lupins have been fed to sheep in addition to their normal ration. With such a large intake of nutrients both nitrogen and energy balances must be affected in some way and such effects are probably inter-related.

As lupin grain contains a relatively high proportion of protein (28-40%, Hove, 1974), one would expect that supplementary feeding with lupins would make more amino acids available in the general circulation. Teleni *et al.* (1989) suggested that a greater supply of glucogenic amino acids during lupin feeding could be in part responsible for the higher glucose entry rate in such sheep.

Lupin grain therefore provides energy and protein substrates, any of which may be responsible for stimulating reproductive function. If either glucose, volatile fatty acids or amino acids were to be responsible for stimulating testicular growth in lupin-supplemented rams, the potential for improvement in feeding precision for testicular growth should be quite substantial. The interaction of both energy and protein yielding nutrients, the metabolic partitioning of nutrients, the actions of specific nutrients on gonadotrophin secretion, and the neuroendocrine pathways involved in such relationships are unclear and represent a rich area for future endeavour.

The effects of supplementary feeding of reproduction in the ram (The 'lupin effect')

The stimulation of gonadotrophin secretion and testicular growth in lupin-fed animals was observed in many experiments and is then highly repeatable (Boukhliq, 1993). There are three types of responses to supplementary feeding, the first of which is the stimulation of pulsatile LH secretion. This response is expressed as a 2 to 3 fold increase in LH pulse frequency and can be obtained after a period as short as 2-3 days, but thereafter it declines and becomes undetectable after 3-4 weeks. The second response is that of FSH which usually starts to be detectable 1 week to 10 days after supplementation has started and is maintained for several weeks. The third response, an increase in testicular size is rarely detectable before 2 weeks but continues for at least 5 weeks (Boukhliq, 1993).

Supplementary feeding and the potential sites for the action within the reproductive system

The effect of differential feeding on reproduction of mature rams was studied by Oldham *et al.* (1978). Rams fed at high level showed significant increase in testicular volume and body weight. These results led to the conclusion that testicular volume is more sensitive to changes in diet than body weight indicating a preferential allocation of nutrients to the reproductive system. It was also observed that a good relationship exists between nitrogen balance and testicular growth in response to lupin feeding, suggesting that protein may be the most important nutrient in adult rams.

Direct effect on the hypothalamo-pituitary axis (GnRH-dependent pathways)

The evidence surrounding the mechanism by which nutrition affects reproductive activity in rams appears to be contradictory. The hypothesis that nutritionally-induced changes in testicular size are a result of changes in the frequency of LH pulses was rejected by Martin *et al.* (1987) who found that supplementing rams with lupins for 9 weeks did not have a significant effect on the secretion patterns of LH and testosterone nor on the responsiveness to GnRH or hCG. Moreover, they concluded that the increase in testicular size was independent of the degree of response of the testis to LH. In another experiment, Ritar *et al.* (1984) reported that lupin supplementation increases the frequency of LH pulses 5 days after feeding but the effect disappeared over the following 6 weeks. In a more recent study, Martin *et al.* (1989) measured LH pulse frequency over a range of times after changing the diet. They found that changing the diet from maintenance to maintenance plus lupins increased LH pulse frequency over the first 3-4 weeks but thereafter the difference between these dietary treatments disappeared (Martin *et al.*, 1990; Martin *et al.*, 1992). These observations agree with those by Ritar *et al.* (1984) and Lindsay *et al.* (1984) and explain those by Sutherland and Martin (1980) who failed to detect an effect of diet on LH pulse frequency because their first measurement was too late (i.e., 9 weeks after change of diet).

An alternative to the direct effect of nutritional stimuli on the pulse generator itself is an effect on the negative feedback system by testicular hormones to change the responsiveness of the hypothalamus to testicular hormones. It is possible that changes in metabolic clearance rate rather than changes in secretion rates can have a potential effect of blood concentrations of reproductive hormones. Dietary changes affect the activity of the liver, the site of catabolism of many hormones and a high plane of nutrition could result in increased clearance or testosterone by the liver leading to a decrease in circulating concentrations that could alter the secretion of gonadotrophins and hence testicular activity in the ram.

Direct effect on the testis (GnRH-independent pathways)

In mature Merino rams, active immunization against GnRH resulted in testicular regression. However, this effect was delayed by a high plane of nutrition (Hötzel *et al.,* 1992), suggesting that the effect of nutrition on testicular growth in rams is partially independent of changes in gonadotrophin secretion. Such responses may involve metabolic hormones such as insulin, insulin-like growth factor-I, growth hormone and prolactin which have been shown to have testicular receptors and can act by promoting cell replication and steroid production (Saez *et al.,* 1988). These metabolic hormones could modulate the effect of gonadotrophins on the testis through promoting the synthesis of one or several hormones produced within the testis as suggested by Martin *et al.* (1992). We have yet to understand the gonadotrophic effects of these metabolic changes in the ram.

Endocrinology of the 'lupin effect' in the ram

In lupin-fed animals, the changes in live weight and testicular growth induced by supplementary feeding are not always accompanied by changes in gonadotrophin secretion. In fact, the three fold increase in LH pulse frequency observed in the first week of supplementary feeding is considerably reduced by week 5. At the same time, the testes keep growing despite a reduction in gonadotrophin secretion especially LH (Boukhliq *et al.*, 1993). This suggest that the testes are capable of growing with minimal or absent gonadotrophic support. Such finding challenges our understanding that testicular growth is solely driven by gonadotrophins (Lincoln *et al.,* 1977; Courot *et al.,* 1979; Lincoln, 1979; Jeffcoate *et al.,* 1982) and indicate that somatotrophic and gonadotrophic axes may probably interact to regulate testicular growth. It is possible that under some circumstances, testicular growth could be more sensitive to changes in the somatotrophic axis, and less sensitive to changes in the gonadotrophic axis. In other words, factors that are associated with the regulation of somatic growth could exert more important and direct effects on the testis than gonadotrophins.

The precise nature of signals encoded by the testis for stimulation of its growth is unknown and our data suggest a permissive, rather than a regulatory role for gonadotrophins (mainly LH) in the testicular response to improved nutrition in Merino rams. Thus, the available data add another degree of complexity to what has been termed the *'lupin effect'* by endorsing a role for gonadotrophin-dependent and -independent pathways in the nutritional control of testicular growth. These pathways could be dissociated by inhibiting or neutralizing endogenous GnRH using immunization against GnRH or hypothalamo-pituitary disconnection in association with nutritional treatments.

Another question which remains unanswered is whether the fast LH pulse frequency in lupin-fed rams results from a stimulation of GnRH release, or from the deactivation of an inhibitory system. The role of endogenous opioids in mediating this response remains controversial, though recent studies from our laboratory indicate that the effect of nutrition is not mediated through the opioidergic system as treatment with naloxone, an opioid receptor antagonist, increased LH secretion in rams on a high plane of nutrition, but not in those on a low plane (Miller *et al.,* 1992).

Conclusion

The lupin-supplemented ram, thus, provides an extremely powerful model for studies of the interface between nutrition and the reproductive neuroendocrine function. Because of its ability to produce fast LH pulse frequency and large FSH concentrations over a short period, lupin feeding represents a sensitive system (*model*) that could be used to explore changes in metabolic status and attempt to reproduce the reproductive response by targeting a single or a group of metabolites at a time.

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