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# Dietary protein or energy restriction both delay age at puberty in ewe lambs†

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**SUMMARY -** The working hypothesis was that restricted protein content in diet of prepubertal ewe lambs would delay time of onset of puberty to a greater extent than restricted energy content. Restriction of dietary protein (Metabolizable Protein: MP) or Energy (Metabolizable Energy: ME) on age and weight at puberty were assigned to one of the three dietary treatment groups: control (CTL; 18 MJ ME $d^{-1}$ and 173 g MP $d^{-1}$); protein restricted (PR; 11 MJ ME $d^{-1}$ and 66 g MP $d^{-1}$); energy restricted (ER; 10.2 MJ ME $d^{-1}$ and 96 g MP $d^{-1}$). Ewe lambs were weighed bi-weekly and bled weekly, and serum was assayed for progesterone to determine time of puberty. Lambs in the CTL group gained more than lambs fed the PR diet and lambs fed the PR gained more weight than ER group ($P<0.05$). Diet affected age ($P<0.001$) but not weight ($P=0.13$) at puberty. Body weight gain prepuberty is more important than type of dietary restriction (protein or energy) with regard to impacting age at puberty. We therefore reject the working hypothesis that dietary protein restriction prepuberty would have a greater impact in delaying onset of puberty than dietary energy restriction.

**Key words:** Sheep, dietary restriction, protein, energy, puberty.

***RESUME -*** *"La restriction en protéine alimentaire ou en énergie retardent toutes deux l'âge à la puberté des agnelles". L'hypothèse de travail a été qu'une teneur restreinte en protéine dans le régime d'agnelles pré-pubères retarderaient l'apparition de la puberté dans une plus grande mesure qu'une teneur restreinte en énergie. La restriction de protéine alimentaire (Protéine Métabolisable : PM) ou d'énergie (Energie Métabolisable : EM) sur l'âge et le poids à la puberté a été assignée à l'un des trois groupes de traitement alimentaire : témoin (CTL ; 18 MJ EM $d^{-1}$ et 173 g PM $d^{-1}$) ; avec restriction en protéine (PR ; 11 MJ EM $d^{-1}$ et 66 g PM $d^{-1}$) ; avec restriction en énergie (ER ; 10.2 MJ EM $d^{-1}$ et 96 g PM $d^{-1}$). Les agnelles étaient pesées deux fois par semaine et subissaient une prise de sang une fois par semaine, et le sérum a été testé pour la progestérone afin de déterminer la période de la puberté. Les agnelles du groupe CTL avaient plus de gain que les agnelles recevant le régime PR, et les agnelles alimentées avec le régime PR gagnaient plus de poids que le groupe ER ($P<0,05$). Le régime a affecté l'âge ($P<0,001$) mais pas le poids ($P=0,13$) à la puberté. Le gain de poids corporel avant la puberté est plus important que le type de restriction alimentaire (protéine ou énergie) en ce qui concerne l'impact sur l'âge à la puberté. Nous rejetons donc l'hypothèse de travail selon laquelle la restriction en protéine alimentaire avant la puberté aurait un impact plus grand sur le retard de l'apparition de la puberté, que la restriction en énergie alimentaire.*

***Mots-clés :*** *Ovins, restriction alimentaire, protéine, énergie, puberté.*

## Introduction

One way of enhancing reproductive performance of the female sheep is through extending its lifetime productivity. It is recognized that age at puberty and age at first lambing are important traits concerning overall reproductive performance. While the endpoint used to define puberty occurs at a discrete point in time, the process of sexual maturation occurs gradually during the pre-, peri- and postpubertal periods in the female. Studies of physiological processes relating to puberty in a number of species have provided evidence for the importance of dietary intake on sexual maturation. Hafez (1952) suggested that a minimum body weight was critical for first ovulation to occur in ewe lambs. Lambs reared on a high plane of nutrition had increased uterine and pituitary weight and more marked thyroid development (Allen and Lamming, 1961). However, there is no clear effect of a single

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nutritionally related blood metabolite on processes of sexual maturation (Schillo, 1992). Different factors (body fat, insulin, non-esterified fatty acids, amino acids, and availability of metabolic fuel such as glucose) may interact to regulate secretion of hormones that modulate age at puberty.

In previous studies where the influence of restriction of dietary content of protein energy on reproductive processes have been compared, protein status has been defined in terms of crude or digestible protein. Protein deficiencies in ruminants are frequently accompanied by altered ruminal energy metabolism. Restriction of protein intake should then be established in terms of metabolizable protein rather than in crude or digestible protein to avoid confounding of energy and protein restrictions (Drouillard *et al.,* 1991).

The working hypothesis in the present study was that diets restricted in protein would delay onset of puberty in lambs to a greater degree than energy restriction. The present experiment was, therefore conducted, to evaluate effects of metabolizable protein on energy restrictions on body weight gain, age at puberty and weight at puberty.

## Materials and methods

### Animals and dietary content

The experiment was conducted on 50 prepubertal ewe lambs that were stratified by weight within genotype and assigned to one of three groups: 1. control; 2. low energy; and 3. low protein. Eighteen ewe lambs were white face (Finn Landrace crosses) and the remaining were black face (Suffolk and Hampshire). The pelleted diets were group fed in an indoor pen once daily to achieve a daily gain of 220 g in the control group and 110 g in both restricted groups. The groups had adequate bunk space for all ewes to consume feed at the same time. At the start of the experiment (late June, 1991) animals weighed 35 kg (range: 30.6-41.8), 35.5 kg (29.7-43.6), and 35.5 kg (31.9-45) in the control, low energy and low protein group. They were 133 d (range: 123-145), 130 d (104-145) and 133 d (123-139) of age, respectively. The ewe lambs were bled weekly and weighed bi-weekly during the 170 d feeding period (June 26 - December 14, 1991). Ewe lambs that initiated estrous cycles were removed from the experiment and feed allowance adjusted downwards for the remaining animals.

### Dietary formulations

Basal portions which consisted of corn cobs, were low in both energy and protein (2.8% CP and 48% TDN). It was therefore possible to prepare diets first-limited in one primary nutrient (i.e., protein or energy) by supplementing the opposite nutrient. The energy restricted diet contained a mixture of feather meal and blood meal (high ruminal escape) to ensure that energy, rather than metabolizable protein was limiting. The blood meal and feather meal were mixed with small amounts of molasses to enhance palatability. Molasses was added to the protein restricted diet as a source of supplemental energy. Urea was also included in quantities sufficient to allow for adequate microbial protein synthesis and growth from energy present from the diet. As a result, metabolizable protein content of the protein restricted diet was limited to that provided by the basal portion of the diet plus that of microbial origin (Table 1).

### Blood sampling

Serum harvested from blood samples was assayed for progesterone. Concentrations of progesterone in serum samples were determined by radioimmunoassay (Roberson *et al.,* 1989). Day of initiation of *corpus luteum* function was used as indicator of attainment of puberty in ewe lambs and was determined based upon the following criteria: concentrations of progesterone $\geq$1 hg $ml^{-1}$ of serum for two consecutive sampling dates. Five ewe lambs, from the restricted groups, did not initiate estrus by termination of the experiment. The latter date was recorded as the puberty date for these animals. Some animals (1 in control, 5 in low energy and 4 in low protein) were removed from the experiment for health reasons, before they attained puberty and their data were not analysed.

Table 1. Composition of diets, % TDN, % CP and % metabolizable protein (MP) on DM basis

| *Ingredients* | *Control* | *Low energy* | *Low protein* |
|---|---|---|---|
| Alfalfa | 46.6 | - | - |
| Soybean hulls | - | 33.7 | 55.9 |
| Corn grain, rolled | 49.4 | - | - |
| Corncobs | - | 52.4 | 34.9 |
| Feather meal | 0.3 | 2.4 | - |
| Blood meal | 0.3 | 2.4 | - |
| Urea | 0.2 | 1.0 | 1.0 |
| NaCl | 0.26 | 0.27 | 0.27 |
| Dicalcium phosphate | 0.13 | 0.85 | 0.82 |
| TM mineral† | 0.04 | 0.04 | 0.04 |
| Se premix†† | 0.01 | 0.01 | 0.01 |
| Molasses | 2.6 | 6.9 | 6.9 |
| DM intake††† (kg) | 1.59/1.69 | 1.12/1.17 | 1.12/1.17 |
| % TDN | 75 | 60 | 65 |
| % CP | 15.0 | 13.5 | 11.4 |
| % MP†††† | 10.9 | 8.6 | 5.9 |

†10% Mg, 6% Zn, 4.5% Fe, 2% Mn, 0.5% Cu and 0.3% I
††0.06% Se
†††Indicates an adjustment in intake on October 8th, 1991. Starting that same day, the restricted ewe lambs received an additional 0.11 kg head$^{-1}$ d$^{-1}$ of soybean hulls (85% IVDMD and 5% CP)
††††Calculated (Burroughs *et al.*, 1975)

## Statistical analyses

Lambs assigned to each diet were a mix of two biological types: white face cross and black face cross. The data were analysed by GLM (SAS, 1985) to test the main effects of diet and genotype and their interaction on growth age and weight at puberty.

## Results

The average daily gain in the control group was 3.1 and 2.4 times that of ewes from the low energy and low protein group, respectively (Table 2). The analysis of variance showed that black face recorded a higher gain than white face ewe lambs (Table 3).

Table 2. Initial age and weight, gain, and weight and age at puberty of lambs fed the three diets

| Variable | Control | Low energy | Low protein | SEM† |
|---|---|---|---|---|
| Number of lambs | 19 | 15 | 16 | |
| Initial age (d) | 132 | 130 | 133 | 1.9 |
| Initial BW (kg) | 35.0 | 35.5 | 35.5 | 0.7 |
| ADG (g/d) | 197$^{a}$ | 62$^{b}$ | 81$^{c}$ | 10 |
| Weight at puberty (kg) | 46.8 | 44.1 | 44.3 | 1.4 |
| Age at puberty (d) | 205$^{a}$ | 259$^{b}$ | 242$^{c}$ | 5 |

†Pooled standard error of the mean
a,b,c: Means within the same row with unlike superscript differ P<0.05

Table 3. Initial age and weight and gain in the two biological types[†]

| Variable | White face | Black face | SEM[††] |
|---|---|---|---|
| Number of lambs | 18 | 32 | |
| Initial age (d) | 129[a] | 134[b] | 1.5 |
| Initial BW (kg) | 33.7 | 36.0 | 0.6 |
| ADG (g/d) | 112[a] | 121[b] | 8 |
| Weight at puberty (kg)[†††] | 43.1[a] | 46.9[b] | 1.2 |
| Age at puberty (d)[†††] | 224[a] | 246[b] | 4 |

[†]White face = Finn Landrace crosses: Finnsheep is a prolific sheep, early puberty, mature weight is 49-67.5 kg); black face = Suffolk and Hampshire: both breeds are used in terminal crosses; Hampshire: mature weight is 78.7-112.5 kg; Suffolk: mature weight is 90-135 kg and is largest breed in USA (Sheep Production Handbook, 1988)
[††]Pooled standard error of the mean
[†††]Data adjusted for initial weight
a,b: Means within row the same row with unlike superscript differ P<0.05

There was no interaction between diet and breed type on age and weight at puberty. Diet affected age but not weight at puberty. Control ewe lambs reached puberty 54 and 37 d earlier (P<0.001) than lambs fed low energy and low protein diets, respectively. Likewise, lambs fed low protein reached puberty earlier than lambs fed the low energy diet (P<0.05).

Genotype had an effect on both age (P<0.001) and weight (P<0.05) at puberty. The white faced ewe lambs had a slower rate of gain, attained puberty 22 d younger and 3.8 kg lighter than the black faced ewe lambs.

The dietary effect appeared 7 weeks after the start of the experiment, when control lambs initiated estrous cycles. Lambs in the low protein group started to attain puberty 4 weeks after the control ewes and ewes in the low energy group started to attain puberty 4 weeks after those of low protein fed group.

A subset of 10 ewe lambs with similar ADG from both energy and protein restricted groups were analysed with the same statistical model showed that both age (P=0.51) and weight (P=0.62) at puberty were not affected by the type of restriction.

## Discussion

Diet had an effect on age at puberty but not on weight at puberty. This is in agreement with Hafez (1952) and Dyrmundsson (1973) who, first suggested the 'minimum body weight' concept for ewe lambs. However these results are not consistent with the findings of other authors (Allen and Lamming, 1961; Burfening *et al.,* 1971; Keane, 1975; Quirke, 1979) who demonstrated that ewe lambs growing at faster rates exhibited their first estrus and were most likely to conceive at a younger age and heavier body weight than ewe lambs growing at slower rates. It could be hypothesized in the present study that there is a minimum weight and/or body weight gain above which dietary restriction would not affect weight at puberty. Although not addressed in the present experiment, body composition may have been different for similar weights among dietary groups (Drouillard *et al.,* 1991).

Concomitantly, the present experiment clearly demonstrated that genotype affected both age and weight at puberty. Thus black faced ewe lambs (ram/meat breed) were heavier and older at puberty than the white faced lambs (ewe/maternal breed). Large number of references for breed differences are reported in the literature (Hafez, 1952; Dyrmundsson, 1973; Quirke, 1977; Quirke *et al.,* 1985). Wiltbank *et al.* (1966) reported that greater than half of the effect of genotype in age at puberty in heifers was present after accounting for pre- and post-weaning average daily gain. The greater

proportion of white faced ewe lambs that attained puberty may be due to a longer breeding season of this genotype.

It is recognized that the effect of nutrition on attainment of puberty is mediated through diminished luteinizing hormone (LH) release (Swanson *et al.,* 1972). Low dietary energy intake prolonged the negative effects of estradiol on release of LH, and response to negative feedback regulation of LH release declined after initiation of feeding a high energy diet (Foster *et al.,* 1986). Inadequate nutrition inhibits reproduction by actions exerted on hypothalamic neurons responsible for release of LHRH (Ebling *et al.,* 1990; I'Anson *et al.,* 1990). Schillo (1992) concluded, that there is no clear effect of a single metabolite on release of LH from the pituitary gland.

## Conclusion

The relative importance of protein or energy deficiencies on puberty attainment can not be clearly concluded from this study. Prepubertal body weight gain is a good indicator of age and weight at puberty. This leads to speculation that the law of 'first limiting nutrient' not only applies to weight gain but also impacts on reproductive development in ewe lambs. If either deficient amounts of protein or energy in diets results in decreased rate of body weight gain, onset of puberty may be delayed. We, therefore, reject our working hypothesis that dietary protein restriction prepuberty would have a greater influence on delaying age at puberty in ewe lambs than restriction of dietary energy intake. Genotypic differences are important to capitalize on, in replacement ewe lambs management.

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