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**Original article**

# Influence of nutrition on testicular growth in Corriedale rams during spring

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**Abstract** — To study the effect of nutrition on spring testicular growth, four adult Corriedale rams were allowed to graze enough to maintain weight (maintenance group), while another four rams, in addition to forage, received a supplemental grain-based ration (increased gradually from 100 to 400 g during the first 5 d and kept at 400 g thereafter) daily for 63 d (supplemented group). Body weight, scrotal circumference, inguinal hyperaemia and testicular consistency were assessed. Blood concentrations of LH and testosterone were measured for 24 h on the day before supplementation began, the day after the animals were fed 200 and 400 g, and 12 and 28 d after animals began to receive the supplement. On these occasions blood contents of non-esterified free fatty acid and β-hydroxybutyrate were measured when animals were fasting. Supplemented feeding increased body weight within 21 d and scrotal circumference within 35 d ($P < 0.01$). Scrotal circumference also increased in rams of the maintenance group ($P < 0.01$) but a lower rate than the supplemented group ($P < 0.001$). In both groups, testicular consistency and inguinal hypereamia increased ($P < 0.01$). In the supplemented group a transient increase ($P < 0.01$) in LH pulsatility occurred the day after rams had received the full supplement (400 g) and 5 d later (day 12). However, no difference was found in total testosterone release between groups. In conclusion, improved nutrition accelerated the testicular growth in spring, although only a transient increase in LH pulsatility was observed. The scrotal circumference of rams kept on maintenance diet did also increase, which indicates that nutrition is not the only environmental cue responsible for the vernal testicular redevelopment in Corriedale rams.


**ram / testicular growth / LH / nutrition / Corriedale**

**Résumé — Influence de la nutrition sur la croissance testiculaire au printemps chez les béliers Corriedale.** Afin d'étudier l'interaction de la nutrition avec la croissance testiculaire observée au prin-

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temps chez le bélier. 8 béliers adultes Corriedale ont été répartis en deux groupes ($n$ = 4/groupe). Les animaux du premier groupe pâturaient pour maintenir leur poids corporel (lot maintenance) ou étaient supplémentés par du concentré (400 g/j à partir du 5e j) pendant 63 j (lot supplémenté). Le poids corporel, la taille et la consistance testiculaire, les concentrations plasmatiques de LH et testostérone et certains métabolites sanguins (acides gras non estérifiés, β-hydroxybutyrate) étaient suivis à quatre étapes du traitement nutritionnel (avant, pendant la période de transition, 12 puis 28 j après l'initiation de la supplémentation). Les poids corporel et taille testiculaire commençaient à diverger dès 21 et 35 j respectivement de supplémentation, et ceci bien que la taille testiculaire augmente également dans le lot « maintenance ». La secrétion pulsatile de LH était supérieure dans le lot « supplémenté » ($p < 0,1$) pendant la période de transition et à j12. En revanche, les deux groupes présentaient une production de testostérone identique. Les auteurs concluent que la croissance testiculaire observée au printemps est accélérée par une alimentation améliorée alors que la pulsatilité de LH n'est augmentée que transitoirement. La taille testiculaire des béliers du lot « maintenance » augmentant également, montre que la nutrition n'est pas le seul facteur impliqué. © Inra/Elsevier, Paris.

**bélier / testicule / LH / androgènes / nutrition**

## 1. INTRODUCTION

Gonadal activity in rams, as in ewes, varies seasonally, although rams never lapse into a period of complete sexual quiescence and infertility. Photoperiod is the main environmental cue governing the annual testicular cycle in rams [9] and modifies the pituitary response to the effect of nutrition [6]. Sensitivity to photoperiod varies among breeds [10]. In Merino rams, nutritional cues seem to be more important than photoperiod, as indicated by reports that nutrition can override the effects of photoperiod [14, 17, 18]. The Corriedale sheep, a Merino-derived breed used in the Southern Hemisphere to produce wool and meat, is a seasonal breeder [19]. Scrotal circumference in Corriedale rams decreases during autumn and increases during spring [20] as it does in Merino rams in similar conditions [21]. In the pastoral system in which Corriedale rams are reared in Uruguay, part of Argentina and southern Brazil, there is an increase in availability and quality of both improved and native pastures during spring [2], which is reflected in an increase in live weight [19–21]. The impact of the natural improvement in nutrition during spring on the seasonal pattern of testicular size is not clear. The objective of this study was to determine whether improved nutrition can stimulate testicular growth in Corriedale rams during spring, when the influence of photoperiod should be inhibitory.

## 2. MATERIALS AND METHODS

### 2.1. Animals, location and treatments

The experiment was carried out from late winter until mid-spring (mid-August to mid-November) at the Faculty of Agriculture, Montevideo, Uruguay (35° SL). During the course of the experiment the daylength increased from 11 to 14 h. Average temperatures during the experiment varied between + 13 and + 19 °C, and monthly rainfall ranged from 30 mm in August to 106 mm during September.

Eight sexually mature, 3-year-old, healthy Corriedale rams weighing 55.0 ± 3.0 kg, with a scrotal circumference of 27.6 ± 1.1 cm (mean ± sd) were used. Animals were shorn 1 month before the experiment began. Animals were reared outdoors, and thus exposed to the natural photoperiod, throughout the experiment. They foraged on strip grazing with annual rye grass (*Lolium multiflorum*), oat (*Avena sativa*) and lucerne (*Medicago sativa*). Daily pasture allowance was 2.5 kg of dry matter/100 kg of live weight to achieve maintenance requirements, and the fenced area was adjusted according to weekly forage availability. Water was provided ad libitum. All animals were managed in a single

group. It was verified that the animals maintained their weight during the first 28 d of the experimental period. The animals were then randomly assigned to two dietary groups. The supplemented group ($n$ = 4) received a grain-based ration (*table I*) once a day at 1000 hours for 63 d. The supplement was given in an individual pail, and the animals were checked daily to make sure that they had eaten the entire grain ration. The animals receiving supplement were started off with 100 g on the first day, followed by 200 g on the second and third days, 300 g on the fourth and fifth days and 400 g on all subsequent days. The maintenance group ($n$ = 4) did not receive supplement and served as a control group. During the 24-h blood sampling periods, animals were placed in small pens (two or three rams per pen) under natural light and fed with forage cut from the same area they had been grazing. The supplement was given in individual pails, as described earlier.

**Table I.** Ingredients and nutrient composition of the supplement.

| Ingredients | |
|---|---|
| Ground sorghum grain (%) | 48.1 |
| Ground corn grain (%) | 50 |
| Calcium carbonate (%) | 0.6 |
| Dicalcium phosphate (%) | 0.8 |
| Trace mineralized salt (%)* | 0.5 |
| Nutrient composition | |
| Dry matter ($g \cdot kg^{-1}$) | 884 |
| Crude protein ($g \cdot kg^{-1}$) | 105 |
| NDF ($g \cdot kg^{-1}$) | 126 |
| ADF ($g \cdot kg^{-1}$) | 53 |
| ME ($MJ \cdot kg^{-1}$ DM)** | 12.1 |

* Composition: 98 % NaCl, 0.35 % Zn, 0.28 % Mn, 0.18 % Fe, 0.035 Cu, 0.0007 % Y, 0.0007 % Co.
** Calculated metabolizable energy.

## 2.2. Measurements and collection of blood samples

Body weight was recorded twice a week, early in the morning before the animals were fed the supplement. Scrotal circumference was measured once a week with a flexible tape at the widest scrotal diameter. At the same time, the bare skin in the abdomen immediately anterior to the scrotum, and on the inside of the hind legs was inspected for signs of hyperaemia, and was rated on an intensity scale of 1–5 using a colour code (pale, pink, red, strong red and purple). The consistency of the testes was assessed by palpation. The testes were graded on a 1–5 scale on the basis of their flaccidity/firmness. All assessments were performed twice by the same two persons for the entire experiment. The measurements of the different variables were highly repeatable both within and between operators.

All rams were bled on five occasions. Cannulae were inserted in a jugular vein 8–10 h before the sampling period began. The first occasion was the day before supplementation began and was defined as day –1. The second occasion was the day after the animals had been fed 200 g of the ration (day 3). The other occasions were the day after the rams had been fed 400 g (day 7), and 5 (day 12) and 21 d (day 28) thereafter. Blood (3 mL) was collected at 20-min intervals for 24 h starting at 2000 hours. Blood samples were immediately centrifuged, and blood serum was harvested and stored at –20 °C prior to the analysis of hormone content.

## 2.3. Hormone analyses

Testosterone contents were determined in samples taken every 2 h in order to estimate the total release per 24 h, using a commercial RIA kit without extraction (Coat-A-Count testosterone; Diagnostic Products Corporation, Los Angeles, CA, USA) previously validated for sheep plasma [19]. The intra-assay coefficients of variation were 15.4 % (4.0 $nmol \cdot L^{-1}$), 5.6 % (22.4 $nmol \cdot L^{-1}$) and 15.8 % (48.8 $nmol \cdot L^{-1}$). The inter-assay coefficients of variation of five assays were 14.6 (4.0 $nmol \cdot L^{-1}$), 8 % (22.4 $nmol \cdot L^{-1}$) and 15.4 (48.8 $nmol \cdot L^{-1}$). The limit of detection (defined as the intercept of maximal binding – 2 SD) was 0.2 $nmol \cdot L^{-1}$. The standard curve and control samples were processed in duplicate, and the unknown samples individually.

The plasma contents of luteinizing hormone (LH) were determined in all samples to estimate the number of pulses per 24 h, using a double-antibody radioimmunoassay previously validated for ovine plasma [5]. Intra-assay coefficients of variation were 6.3 % (3.7 $\mu g \cdot L^{-1}$), 4.3 % (5.5 $\mu g \cdot L^{-1}$), and 6.9 % (10.4 $\mu g \cdot L^{-1}$). Inter-assay coefficients of variation were 11.4 % (3.7 $\mu g \cdot L^{-1}$), 11.6 % (5.5 $\mu g \cdot L^{-1}$) and 6.8 % (10.4 $\mu g \cdot L^{-1}$). The limit of detection was 0.4 $\mu g \cdot L^{-1}$. All samples were processed in duplicate.

Contents of non-esterified free fatty acids (NEFA) and β-hydroxybutyrate (β-HBA) were measured in samples obtained at 0400 and 0500 h,

when animals were fasting. The blood concentrations of NEFA and β-HBA were analysed by enzymatic colorimetric methods using commercial kits (NEFAC, Wako Chemicals Inc., Richmond, USA and β-HBA, Sigma Diagnostics, St Louis, USA). The serum analyses were performed in a computerized multichannel spectrophotometer (Cobas Mira, Hoffman-La Roche, Switzerland). Intra-assay coefficients of variation for NEFA were 5.7 % (0.4 mmol·L$^{-1}$) and 6.4 % (0.8 mmol·L$^{-1}$) and the corresponding inter-assay coefficients of variation 3 % (0.4 mmol·L$^{-1}$) and 2 % (0.8 mmol·L$^{-1}$). The intra-assay and inter-assay coefficients of variation for β-HBA were 3.5 % (0.7 mmol·L$^{-1}$) and 5 % (0.7 mmol·L$^{-1}$).

### 2.4. Data analysis

The weekly averaged values obtained for body weight, scrotal circumference, testicular consistency and inguinal hyperaemia were used. LH baseline concentration was calculated using a skewness method [26]. For each period the mean LH level plus 1 SD was calculated using all the values obtained for each period. After excluding all LH values above the mean plus 1 SD, a new mean and SD were calculated. The procedure was repeated until there were no values above the mean plus 1 SD. An LH peak was defined as a value above the mean plus 2 SD and an LH pulse was defined as at least two consecutive peaks. The pulse amplitude was determined as the highest value associated with an individual pulse. The amount of testosterone released during 24-h sampling periods was estimated by calculating the area under the curve (AUC) according to the formula: $T = \Sigma ((T_i + T_{i+1})/2) \times 2$ h, where $i = 1,... n$ is the sample number.

Data, excluding pre-treatment records (i.e. from day −28 to day 0), were subjected to repeated-measure analysis of variance. The effects of supplement, date and the interaction between supplement and date were studied. The variation among rams within groups (maintenance or supplemented groups) was used as an error term when testing the differences between groups. Individual means were compared using the least significant difference (LSD) test in cases where the main effects of breed, date and interaction between breed and date were significant. For scrotal circumference contrast was used to test a first polynomial regression nested by treatment and to test for differences between coefficients of regression for each treatment. All analyses were performed using SAS 608 [24]; procedures: PROC GLM for the continuous variables and PROC CATMOD for the non-continuous. Data are expressed by mean ± sem.

## 3. RESULTS

### 3.1. Body weight, NEFA and β-HBA

Mean body weights for both groups are shown in *figure 1a*. Supplement ($P = 0.001$), date ($P < 0.001$) and the interaction between supplement and date had significant ($P < 0.001$) effects on live weight. In the supplemented group supplementation positively affected body weight during the first 3 weeks. By day 21 after the supplementation began, rams of the supplemented group were heavier than rams of the maintenance group ($P < 0.01$), and they continued gaining weight until the end of the experiment. The mean body weight of the rams in the supplemented group increased about 16 %, whereas the body weight of the rams of the maintenance group remained constant throughout the experiment.

NEFA and β-HBA levels over the study period are illustrated in *figure 2a, b*. There were no effects of supplement ($P = 0.39$), date ($P = 0.06$), or interaction between supplement and date ($P = 0.33$) on the level of NEFA. The β-HBA level was influenced by supplement ($P = 0.001$) indicating that the overall means between groups were different (supplemented group: 0.77 ± 0.02 mmol·L$^{-1}$; maintenance group: 0.60 ± 0.02 mmol·L$^{-1}$). The interaction between date and supplement was significant ($P < 0.001$). β-HBA concentrations decreased in the maintenance group ($P < 0.01$), whereas the β-HBA levels were significantly increased in the supplemented group at the last sampling occasion ($P < 0.05$).

### 3.2. Scrotal circumference, testicular consistency and inguinal hyperaemia

Scrotal circumference increased in both groups during the experiment (*figure 1b*).

Scrotal circumference was affected by date ($P < 0.001$) and an interaction between supplement and date was found ($P < 0.001$). First order polynomial regressions were found for both groups ($P < 0.001$); however, the increase in scrotal circumference

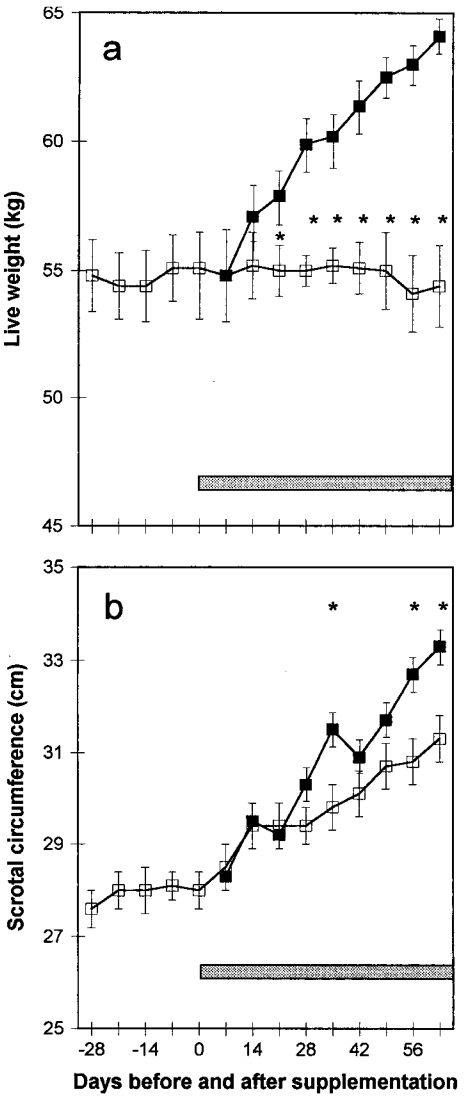


**Figure 1.** Effects of nutrition on the live weight (**a**) and scrotal circumference (**b**) (mean ± sem) of Corriedale rams fed a maintenance diet (□) ($n = 4$) or a diet supplemented with grain-based rations (■) ($n = 4$) during late winter–spring. The shaded bar shows the period of supplementation. Asterisks indicate significant differences ($P < 0.01$).

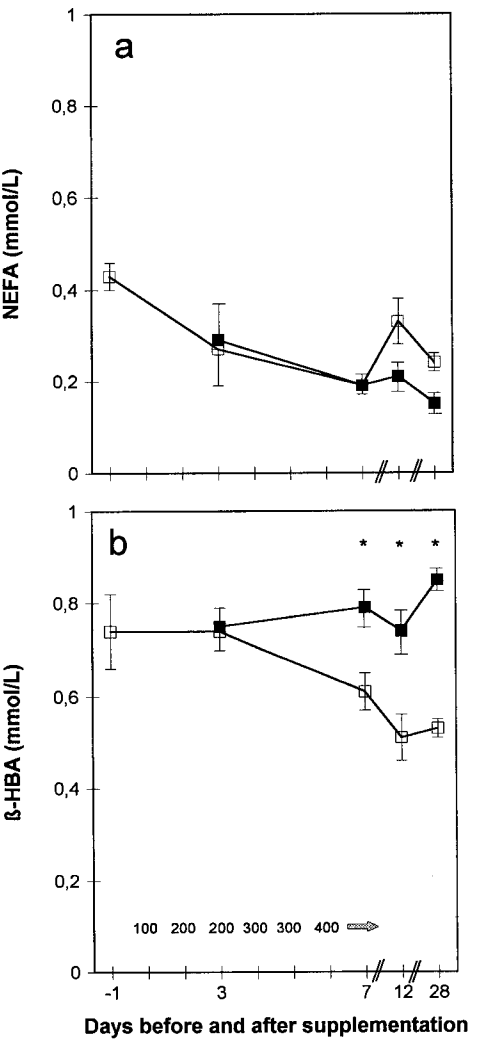


**Figure 2.** The effect of nutrition on levels of non-esterified fatty acids (**a**) and β-hydroxybutyrate levels (**b**) (mean ± sem) in Corriedale rams fed a maintenance diet (□) ($n = 4$) or a diet supplemented with grain-based rations (■) ($n = 4$). Numbers (g) and arrow show how the supplemented diet was given. Asterisks indicate significant differences ($P < 0.01$). Observe the broken X-axis.

was higher in the supplemented group ($\beta = 0.60$) than in the maintenance group ($\beta = 0.31$) ($P < 0.001$). Two weeks after supplementation began, the scrotal circumference of both groups had increased ($P < 0.01$). On day 35 after the supplementation began, animals of the supplemented group showed a larger scrotal circumference than animals of the maintenance group ($P < 0.01$). By the end of the experiment scrotal circumference had increased 12 % in rams of the maintenance group and by 19 % in rams of the supplemented group ($P < 0.01$).

Testicular consistency and inguinal hyperaemia results are shown in *figure 3a, b*. Both variables were affected by date ($P < 0.001$ and $P < 0.01$ for testicular consistency and inguinal hyperaemia, respectively) during the supplementation period. No effect of supplement ($P = 0.81$ and 0.47 for testicular consistency and inguinal hyperaemia,

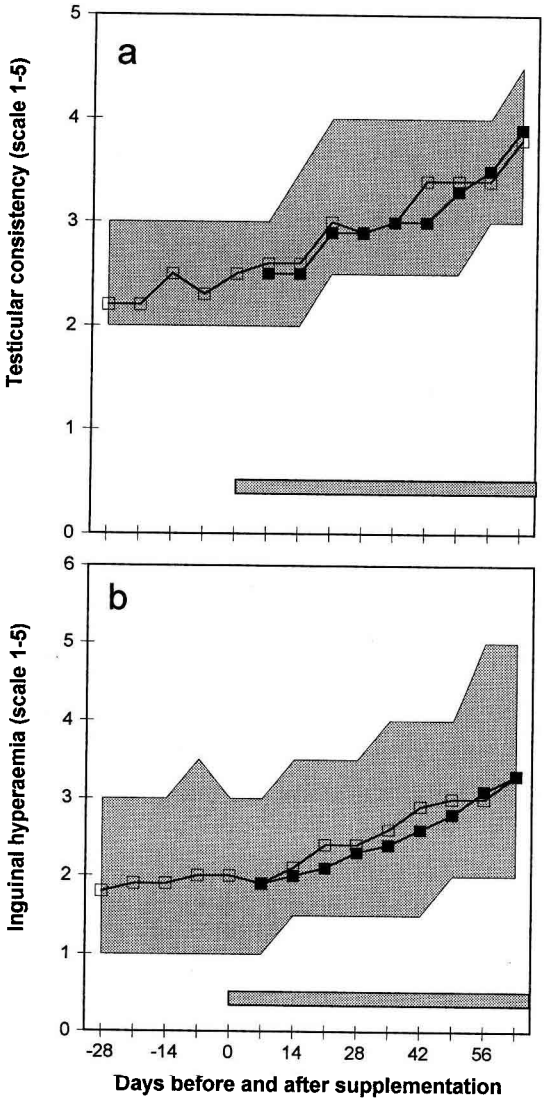


**Figure 3.** The effect of nutrition on testes consistency (**a**) and inguinal hyperaemia (**b**) rated on a 1–5 scale (means and maximum and minimum values) in Corriedale rams fed a maintenance diet (□) ($n = 4$) or a diet supplemented with grain-based rations (■) ($n = 4$). The shaded bar shows the period of supplementation.

respectively) or interaction between supplement and date ($P = 0.94$ and 0.99 for testicular consistency and inguinal hyperaemia, respectively) was found, indicating that changes in testicular consistency and inguinal hyperaemia were similar in the two groups throughout the experiment.

### 3.3. LH and testosterone secretion

#### *3.3.1. LH pulsatility during 24 h*

On day −1, there was no difference in LH pulsatility between groups (Student's *t*-test). Supplementation significantly affected ($P < 0.001$) the number of LH pulses. Animals of the supplemented group showed a higher LH pulse frequency ($6.7 \pm 0.8$) overall than animals in the maintenance group ($4.0 \pm 0.6$) during the supplementation period. LH pulsatility was influenced by date ($P < 0.01$) and by the interaction between supplement and date ($P < 0.01$). Animals in the supplemented group tended to have higher LH pulse frequency than the maintenance group animals on day 3 of the supplementation period ($P = 0.09$). On days 7 and 12, animals in the supplemented group had significantly more LH pulses than animals of the maintenance group ($P < 0.01$) (*figure 4*). By day 28 the LH pulse frequency in the supplemented group had returned to values similar to those in the maintenance group.

#### *3.3.2. LH pulse amplitude*

There was no effect of supplement ($P = 0.80$) or date ($P = 0.20$) on the amplitude of LH pulses, and no interaction between supplement and date was found ($P = 0.95$). LH pulse amplitudes are shown in *figure 4a*.

#### *3.3.3. Total testosterone release (AUC) during 24 h*

No clear difference in total testosterone release was detected.

## 4. DISCUSSION

Improved nutrition increased both body weight and scrotal circumference compared to maintenance diet in Corridale rams during spring. There was a minor but significant increase in scrotal circumference also in the maintenance group, indicating that other factors, in addition to improved nutrition, are involved in the vernal testicular growth in Corridale rams. However, the rate of increase in scrotal circumference was higher in the supplemented group, which means that nutrition can enhance the response to other environment signals, e.g. photoperiod, at this time of the year.

NEFA is an indicator of the nutritional status of animals [25]. The lack of differences between groups in the level of NEFA may be attributed to the short time animals were followed in this experiment. β-HBA concentrations in blood are largely influenced by diet, and levels are higher in animals eating concentrate than in animals eating only forage [25]. The differences observed between groups indicate that the rams of the supplemented group were better fed than animals of the maintenance group. These metabolites were measured as indicators of the nutritional status, since the changes in LH pulsatility were observed before changes in body weight.

Supplemented feeding increased LH pulsatility as soon as animals had received 200 g, although the difference between groups was not significant until the animals had been fed 400 g. The difference in LH pulsatility was no longer evident 3 weeks later, in contrast to differences in body weight and the levels of β-HBA. The lack of difference between groups in LH pulsatility after 21 d of feed supplementation but larger scrotal circumference in rams of the supplemented group supports the hypothesis that a GnRH-independent pathway exists through which nutrition influences testicular growth, as proposed by Martin et al. [15] and Martin and Walkden-Brown [12]. Nutri-

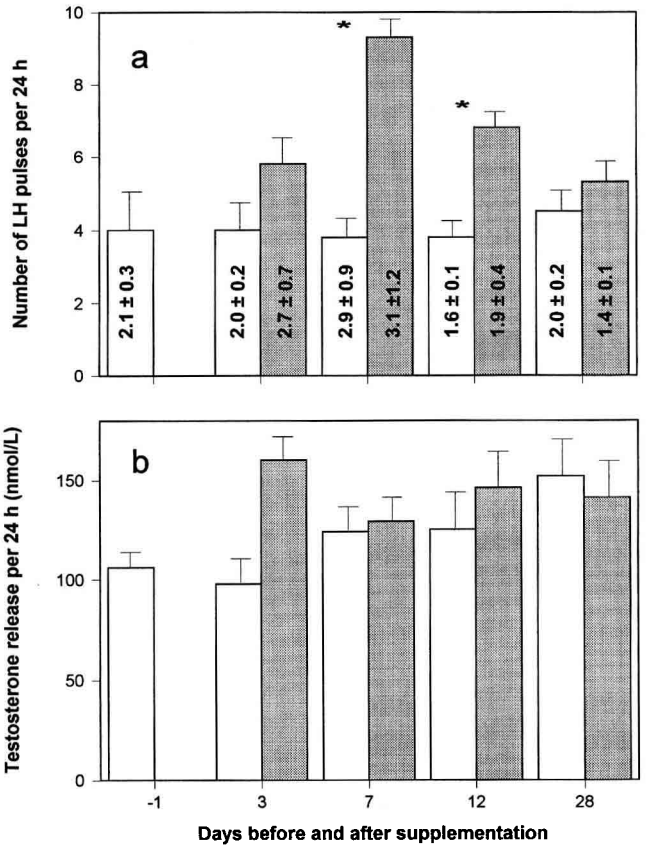


**Figure 4.** The effect of nutrition on number of LH pulses (mean ± sem) (**a**) and total testosterone release (AUC) per 24 h (mean ± sem, arbitrary unit) (**b**) in Corriedale rams fed a maintenance diet (white bars) ($n = 4$) or a diet supplemented with grain-based rations (grey bars) ($n = 4$). Numbers depict mean ± sem of LH pulse amplitude. Asterisks indicate significant differences in LH pulse frequency ($P < 0.01$).

tion seems to have no influence on the amplitude of the LH pulses, as indicated by Martin et al. [16] and our results.

Unexpectedly, a marked decrease in β-HBA in maintenance group from day 7 and only a small increase in the supplemented group were observed. Environmental conditions may give a possible explanation for this. Moderate to heavy rainfalls were recorded the day or days before the three last sampling occasions, which might have had a negative influence on voluntary intake. This could have reduced the consumption of forage, while the concentrate intake was maintained, as the feeding place was protected from rain.

With the method of testosterone measurement we used (total testosterone release per 24 h), some information of testosterone secretion, e.g. pulsatility, is not considered. However, the lack of differences between groups in total testosterone release per 24 h even though the LH pulse frequency increased in the supplemented group is in agreement with the observations made by Martin et al. [13, 16]. This lack of increase in the testosterone response to LH when nutrition increases testicular growth may be

partly explained by the fact that nutrition increases more seminiferous tubular tissue than the interstitial tissue [7]. Changes in the diameter of seminiferous tubules due to improved nutrition have also been observed in Corriedale rams [3]. Other factors may contribute to the lack of increased testosterone response to LH. There is experimental evidence that prolactin promotes steroidogenesis in ram testes [23], but prolactin inhibition in rams did not show a clear influence on testicular size [1, 22]. Prolactin effects on the seasonal ram testicular cycle under normal conditions are limited (see Curlewis [4]), but Lincoln et al. [11] provided evidence from hypothalamo-pituitary disconnected Soay rams, that supported the hypothesis that prolactin may have a stimulatory effect on the testis, acting in long days to prime the responsiveness to gonadotropins, thus permitting the rapid reactivation of the testicular axis in the autumn. When studying Corriedale rams under natural conditions, we found that the levels of prolactin began to increase during early spring (unpublished data).

Testicular consistency and inguinal hyperaemia varied throughout the experiment in the same direction as scrotal circumference. The hyperaemia results suggests that sexual flush has a seasonal pattern in Corriedale rams as has been found in Soay rams [8].

An unexpected finding in this work was that animals of the maintenance group, even though they did not gain weight, increased their scrotal circumference in a way similar to that of Corriedale rams on improved and native pastures during the same period of the year [19, 20].

In conclusion, improved nutrition enhanced the rate of increase of scrotal circumference suggesting that the seasonal increase in availability and quality of forage that occurs in spring in this region may accelerate the testicular redevelopment in Corriedale rams. The transient increase in LH pulsatility in the supplemented group suggests that a GnRH-dependent mechanism is only partially responsible for nutritional effects on testicular growth. Rams kept on maintenance diet also increased their scrotal circumferences, which indicates that nutrition is not the only environmental cue responsible for the testicular growth in spring.

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