Effect of underfeeding on testosterone–LH feedback in the bull

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ABSTRACT
This study was conducted to investigate the hypothesis that the low LH pulsatility induced by underfeeding may result from an altered negative-feedback response to testosterone in cattle.

Eight 21-month-old Aubrac bulls were divided into two equal groups. The first group (group H) received a high level of nutrition (producing a gain in weight of 570 g/day) and the second group (group L) a low level of nutrition (producing a loss in weight of 330 g/day). After 52 days of underfeeding the bulls were hemicastrated, castrated 7 days later and then injected with 30 mg testosterone daily for 14 days, 10 mg daily for 7 days and 2.5 mg daily for 7 days.

After hemicastration, LH pulsatility was higher ($P < 0.001$) in group H (9.0 pulses in 24 h) than in group L (5.3 pulses in 24 h). After castration this value was the same in the two groups (24 pulses in 24 h). When the bulls were injected daily with 30 or 10 mg testosterone, the number of LH pulses in 24 h was lower ($P < 0.05$) in group L (14.3 and 9.8 pulses in 24 h with 30 mg, and 17.3 pulses in 24 h with 10 mg) than in group H (27.8 and 25.5 pulses in 24 h with 30 mg, and 24.0 pulses in 24 h with 10 mg). When the animals received 2.5 mg testosterone daily, the number of LH pulses was the same in both groups (20.3 pulses in 24 h).

These results indicate that the negative-feedback response of the LH-releasing system to testosterone is modified by underfeeding in the bull.

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INTRODUCTION

Undernutrition decreases gamete production in male (Van Demark & Mauger, 1964) and female (Wiltbank, Rowden, Ingalls et al. 1962) cattle. There is substantial evidence which indicates that nutrition affects gonadotrophin levels, underfed cattle having very low plasma follicle-stimulating hormone levels (Gauthier, Terqui & Mauléon, 1983) and weak luteinizing hormone (LH) pulsatility (Gauthier & Berbigier, 1982). In cattle the mechanisms by which diet modulates gonadotrophin secretion are unclear, but they do not seem to be triggered by a decrease in the sensitivity of the pituitary to gonadotrophin-releasing hormone (Gauthier & Mauléon, 1983).

Secretion of LH in female and male rats on a nutritionally low diet is more sensitive to the inhibitory effects of steroids than it is in those on a nutritionally high diet (Howland & Ibrahim, 1973; Pirke & Spyra, 1981). This suggests that sensitivity to the negative-feedback effect of steroids is enhanced by underfeeding in the rat.

The present study set out to test the sensitivity of underfed bulls to testosterone feedback by comparing plasma LH levels of underfed or normally fed animals after hemicastration, castration and injection of different quantities of testosterone.

MATERIALS AND METHODS

Eight 21-month-old Aubrac bulls were divided into two equal groups according to their weights and testicular diameters. They were housed tethered in a stable and fed individually on a diet with either a high (group H) or a low (group L) level of nutrition (Table I). The diets were supplemented with minerals and vitamins according to the recommendations of INRA (1978). The animals were weighed every 7 days (the first day on the low diet was designated day 0).
The bulls were hemicastrated on day 52 and then castrated on day 59. The operations were carried out under light anaesthesia. Thirty mg testosterone, dissolved in propylene glycol:ethanol:benzyl alcohol (6:3:1, by volume), were injected daily from days 75 to 88, 10 mg from days 89 to 95 and 2.5 mg from days 96 to 102.

Blood samples were taken every 20 min for 12 h (07.00–19.00 h) on days −7 and 51, and every 20 min for 8 h (07.00–15.00 h) on days 54, 57, 63, 81, 87, 94 and 101. Plasma LH was assayed in each sample and plasma testosterone assayed in samples taken every 2 h on days 81, 87, 94 and 101. Luteinizing hormone was measured in duplicate in a double-antibody radioimmunoassay (Pelletier, 1972), modified as described by Pelletier, Garnier, de Reviers et al. (1982). Results are expressed as µg CNRS-LH-M3 reference preparation/l. The sensitivity of the assay was 0.2 µg/l. The intra-assay coefficient of variation was 10% for values of B/B₀ from 15 to 70%, measured in ten replicates of two reference samples (0.6 and 5.0 µg/l). Testosterone was measured in duplicate in a double-antibody radioimmunoassay (Garnier, Cotta & Terqui, 1978) after extraction by ethyl acetate: cyclohexane (50:50, v/v). The sensitivity of the assay was 1.39 nmol/l. The intra-assay coefficient of variation was 15% for values of B/B₀ from 30 to 80%, measured in ten replicates of two references samples (5.5 and 70 nmol/l).

Two criteria were used to identify an LH pulse. First, the LH level at the peak had to exceed the 95% confidence limits of the concentration at both the preceding and subsequent nadirs. Confidence limits for the LH concentration in each sample were determined using the method of Huet (1984). Secondly, the amplitude of each pulse, defined as the LH concentration at the peak minus that at the preceding nadir, had to be greater than the sensitivity of the assay.

The number of LH pulses, adjusted to a 24-h period, were compared between groups by the Mann–Whitney rank sum test (Snedecor & Cochran, 1971).

### Table 1. Composition and nutritional value of the diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>Ingredients</th>
<th>Weight (kg dry matter)</th>
<th>New energy (J)</th>
<th>Crude protein: nitrogen × 6.25 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>High level</td>
<td>Hay</td>
<td>7.00</td>
<td>27137</td>
<td>385</td>
</tr>
<tr>
<td></td>
<td>Maize grain</td>
<td>2.50</td>
<td>21322</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>Soya</td>
<td>0.61</td>
<td>4774</td>
<td>270</td>
</tr>
<tr>
<td></td>
<td>Urea + molasses</td>
<td>0.10</td>
<td>460</td>
<td>140</td>
</tr>
<tr>
<td>Low level</td>
<td>Straw</td>
<td>5.00</td>
<td>14630</td>
<td>140</td>
</tr>
<tr>
<td></td>
<td>Maize grain</td>
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<td>8749</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>Urea + molasses</td>
<td>0.10</td>
<td>460</td>
<td>140</td>
</tr>
</tbody>
</table>

![Figure 1](chart1.png)  
**Figure 1.** Liveweights of bulls receiving a high (53693 J net energy intake) (●) or a low (23839 J net energy intake) (▲) level of nutrition. The first day on the low-nutritional diet was designated day 0. There were four bulls/group.

![Figure 2](chart2.png)  
**Figure 2.** Plasma testosterone concentrations in steers (n = 8) injected i.m. with 30 (■), 10 (▲) or 2.5 (●) mg testosterone dissolved in propylene glycol:ethanol:benzyl alcohol (6:3:1, by volume). Four of the eight steers were underfed but, as testosterone levels were not different between the two diets, only the mean values for all the animals were plotted.
FIGURE 3. Variations in (a) the basal level of LH and (b) the number of LH pulses in 24 h in bulls receiving a high (53693 J net energy intake; stippled bars) or a low (23839 J net energy intake; open bars) level of nutrition. There were four bulls/group. The first day on the low-nutritional diet was designated day 0. Animals were hemicastrated on day 52 and castrated on day 59. They were then injected with 30 mg testosterone daily from days 75 to 88, 10 mg testosterone daily from days 89 to 95 and 2.5 mg testosterone daily from days 96 to 102.
The nadirs were analysed by split-plot analysis of variance (Gill & Hafs, 1971).

RESULTS

Weight
At the beginning of the experiment the mean weights were 543 and 540 kg for bulls in groups H and L respectively. After 3 weeks (day 23) of adaptation to the diet the weights were 556 and 543 kg, and at the end of the experiment (day 102) the weights were 601 and 517 kg for groups H and L respectively (Fig. 1). The mean daily weight variation between days 23 and 102 was +570 g/day for group H and −330 g/day for group L.

Testosterone levels
The mean variations in testosterone levels during the days of blood sampling are shown in Fig. 2. There was no significant difference in testosterone levels between the two diets.

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increased in the two groups and there was no difference between the groups (24 pulses in 24 h).

After injection of 30 mg testosterone daily, the number of LH pulses was lower ($P<0.05$) in group L (14·3 and 9·8 pulses in 24 h on days 81 and 87 respectively) than in group H (27·8 and 25·5 pulses in 24 h). After injection of 10 mg testosterone daily, the pulse frequency in group L increased to 17·3 in 24 h but remained unchanged in group H (24·0 in 24 h). The difference between the two groups was not significant ($0.05 < P < 0.1$). After injection of 2·5 mg testosterone daily, the number of LH pulses was similar in both groups (group L, 19·5; group H, 21·0 pulses in 24 h).

The number of LH pulses in 24 h was highly correlated ($r=0·98; P<0·01$) with the weight variation as a percentage of body weight for the animals in group L.

**DISCUSSION**

These data support the observations from previous studies with bulls and rams which have demonstrated that underfeeding decreases LH pulsatility (Gauthier & Berbigier, 1982; Lindsay, Pelletier, Pisselet & Courot, 1984). In the present study an increase of LH pulsatility occurred in the control group immediately after hemicastration, as in rams (Hochereau de Reviers, Loir & Pelletier, 1976). The very slight increase in LH pulsatility after hemicastration in the underfed animals, compared with the control group, shows that the action of underfeeding on the hypothalamo-pituitary axis (HPA) causes either an increase in sensitivity to testicular inhibition or a decrease in the activity of the HPA induced by undernutrition and independent of the testicular action. As LH pulsatility increases normally after castration, whatever the level of nutrition, it is reasonable to assume that the action of underfeeding was not independent of the testis. If it were, then the low LH pulsatility of intact underfed bulls would be due to hypersensitivity of the HPA to gonadal secretions, as has already been described in rats (Howland & Ibrahim, 1973; Pirke & Spyra, 1981). Bulls in group H were not sensitive to the feedback action of testosterone, as indicated by the fact that the number of LH pulses were not different before and during testosterone injections. Underfed animals were very sensitive to testosterone and this sensitivity increased with the degree of undernutrition, as shown by the observation that with the same dose of testosterone (30 mg) the number of LH pulses in 24 h decreased with the duration of underfeeding and the amount of weight lost. As testosterone injections in the present experiment were more efficient in decreasing LH pulsatility in underfed than in normal fed steers, without any difference in testosterone plasma levels, this hormone could be responsible for the low LH pulsatility in underfed entire bulls (Gauthier & Berbigier, 1982).

Leydig cells are able to produce testosterone even under conditions of undernutrition, as shown in Creole bulls (Gauthier & Berbigier, 1982). This testosterone and the hypersensitivity of the HPA to its action would be sufficient to lead to the low gonadotropin levels encountered in underfed animals. This finding could be explained by the lower metabolic rate of this steroid in starved animals, as demonstrated in man (Boyar & Bradlow, 1977). In the present experiment, however, the circulating testosterone levels were the same for all treatment groups. Thus another hypothesis for the hypersensitivity to steroid feedback must be tested in underfed cattle.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


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