

## NUTRITION OF RAMS AND OUTPUT OF SPERMATOZOA

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## SUMMARY

To determine the time taken for the output of spermatozoa by rams to respond to changes in the diet semen was collected twice daily for 63 days from two groups of rams; one received a high and the other a low plane of nutrition. The output of spermatozoa by rams in the two groups began to diverge at the seventh week of differential nutrition, whereas testicular size diverged after four weeks. In contrast, both the live weights and the volume of semen collected began to diverge after one week of semen collection. For all of these parameters the mean values rose for the rams on the high plane of nutrition and fell for those on the low plane of nutrition and differed significantly between the two groups by the end of nine weeks. Because changes in the output of spermatozoa were first evident during the seventh week after changing the diets, it was concluded that the efficiency of stages of spermatogenesis that occur later than the last spermatogonial divisions are responsive to nutrition.

Keywords: Nutrition, Lupin, Spermatozoa, Output.

## INTRODUCTION

Daily production of spermatozoa by rams is sensitive to the dietary intake of protein and energy (Braden et al. 1974; Oldham et al. 1978). The testicular size of rams can be increased before joining (Lindsay et al. 1978) but what period elapses between the time of onset of supplementary feeding and the time at which the rate of output of spermatozoa by rams is increased? In the experiment of Oldham et al. (1978), testicular size appeared to change within one week of the diets of the rams being altered. A greater period probably elapses **before** the output of spermatozoa increases and this will depend on the stages of spermatogenesis that are affected by nutrition. For example, any effects on the divisions of the early type A spermatogonia may take up to 49 days to affect the number of mature spermatids in the testis (Ortavant 1959) and a further 11 days are then required for these to travel through the epididymides of rams ejaculating regularly (Amir and Ortavant 1968). Thus an increase in the number of spermatozoa ejaculated may not be expected until about 60 days after supplementary feeding begins. If, however, the efficiency of later stages of spermatogenesis are affected by nutrition then a more rapid change in the **output** of spermatozoa might be expected. For example, if nutrition affects meiotic divisions, which have previously been shown to be influenced by the **photoc** environment of rams (Ortavant 1956), then the number of spermatozoa **ejaculated may** change within four weeks of the start of supplementary feeding (Ortavant 1959). In our experiment we wished to determine the time taken for the output of spermatozoa by rams to **change** after their diets had been altered.

## MATERIALS AND METHODS

Twenty four, one year old Merino, rams were randomly divided into two groups of 12 after stratifying them on live weight. The rams in Group '1' were fed a ration calculated to increase their liveweights while those in Group 2 were fed a submaintenance ration. Group 1 received 2.1 kg of **concentrate** per ram per day ,

(oats, sweet lupin seed and pellets) and had continual access to **oaten** hay, while Group 2 received 1 kg of **oaten** hay per ram per day. Prior to the onset of differential feeding all the rams were fed **oaten** hay ad libitum. The rams were housed indoors and maintained under natural lighting. The experiment began in early June. Semen was collected from each ram by artificial vagina twice daily for 63 consecutive days. Semen collection began three days before the different groups of rams were placed on their separate diets so as to stabilize their epididymal reserves of spermatozoa before the experiment began. The concentration of spermatozoa of each ejaculate was determined using a calibrated **colorimeter** (Salamon 1976) and the number of spermatozoa ejaculated each day was calculated from the concentration and volume. The rams were weighed weekly before receiving their daily ration. At the same time their testicular weights were estimated by comparing them to a series of calibrated beads (Oldham et al. 1978).

The rate of production of spermatozoa was determined from the numbers of stage VI, VII and VIII spermatids present in testicular homogenates (Amann 1970). Following the 63 days of semen collection the rams were slaughtered and their testicles were excised and weighed. The **tunica albuginea** was then removed and weighed and the testicular parenchyma was placed in a Waring Blender along with 150 ml of diluent and homogenized for 90 seconds. The diluent was 0.09% **NaCl** solution that contained 0.5% **formalin** and 0.05% triton X-100. Each testis was homogenized separately then the two testes were pooled for each ram and the volume made up to 700 ml per 100 g of testis using the same diluent. Cells having the appearance of mature spermatids were counted on a haemocytometer in six samples with four squares per sample. The total number of spermatids was calculated for each ram and divided by 3.56, the number of days production presumed to be represented (Amann 1970). Analysis of variance was used to examine the mean weekly changes in live weight, estimated testicular weight, number of spermatozoa ejaculated and volume of semen collected.

## RESULTS

There was no consistent divergence in the output of spermatozoa from the two groups of rams during the first six weeks after changing the diet (Fig.1). Thereafter the output of spermatozoa from Group 1 progressively rose while that from Group 2 declined. By the end of the ninth week of semen collection the mean daily output of spermatozoa from the rams in Group 1 had increased by  $960 \times 10^6$ . The change in output of spermatozoa from the values obtained in the first week of the experiment varied significantly ( $P < 0.01$ ) between groups of rams and between weeks. The interaction between groups and weeks was also significant ( $P < 0.001$ ).

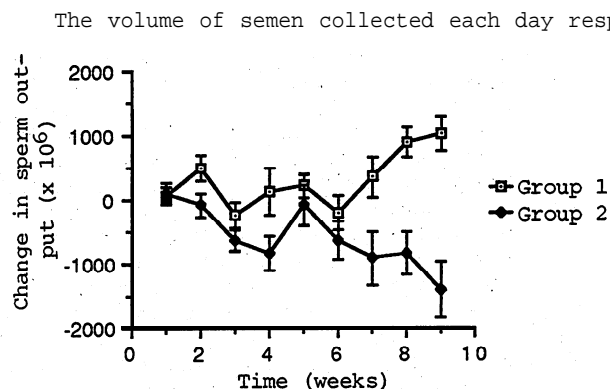


Figure 1 Change in sperm output ( $\times 10^6 \pm \text{SEM}$  over time (weeks))

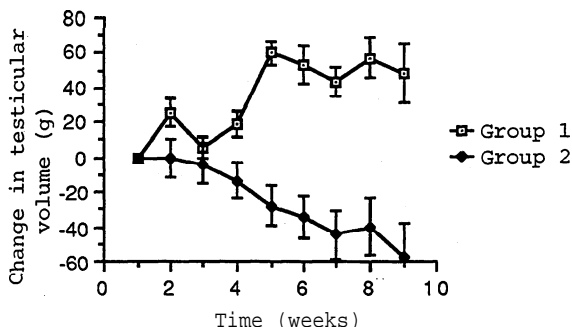
The volume of semen collected each day responded rapidly to changes in the diets, **increasing** almost linearly from the first week from Group 1 and decreasing for Group 2. The analysis of variance revealed that the changes in volume from the values obtained in the first week of the experiment varied significantly between the groups of rams ( $P < 0.01$ ) and weeks ( $P < 0.01$ ) and that there was a significant interaction between groups and weeks ( $P < 0.001$ ).

The estimated testicular

Table 1 The effect of treatment on testicular size and sperm production (mean  $\pm$  SEM)

	Testicular weight (g)	Daily production ( $\times 10^6$ )	Rate of production per gram of testis ( $\times 10^6$ )	Output as % production
Group 1	326.2 $\pm$ 13.7	6,900 $\pm$ 880	22.9 $\pm$ 6.9	57.5 $\pm$ 8.8
Group 2	175.9 $\pm$ 10	2,760 $\pm$ 510	16.8 $\pm$ 5.6	45.2 $\pm$ 3.4

size began to change about three weeks after the diets of the rams were changed. Thereafter, the testicular size of the rams in Group 1 generally increased with time while those of Group 2 decreased. The estimated mean paired testicular size for Group 1 had increased by 50 g while that for Group 2 had decreased by 58 g after nine weeks of the experimental diets.

Figure 2 Change in estimated testicular size ( $\text{g} \pm \text{SEM}$ ) over time (weeks)

progressively lost weight. The rams in Group 1 had gained an average of 17.2 kg while those in Group 2 had lost 5.8 kg by the end of the ninth week and the difference in weight between the two groups was significant ( $P < 0.001$ ).

#### DISCUSSION

The divergence in the output of spermatozoa between the rams on a high plane of nutrition and those on a low plane was evident seven weeks after the nutrition was altered and was probably due, initially, to changes in the efficiency of stages of spermatogenesis that occur later than the 'final spermatogonial division'. This is because it takes about 55 days for changes in the 'efficiency of the late spermatogonial divisions to affect the output of spermatozoa of regularly ejaculated rams (Ortavant 1959; Amir and Ortavant 1968). Changes in the efficiency of the later stages of spermatogenesis have previously been observed in rams in association with changes in their daily photoperiod. Ortavant (1956) found that the transformation of spermatocytes from the zygotene to pachytene stages and the efficiency of meiosis as well as the divisions of the A-spermatogonia were greater in rams exposed to short rather than long days. It is impossible to conclude just which of the later stages of spermatogenesis were affected by nutrition in our experiment, as we do not know how long it took for the testicles of the rams to begin to respond to the changes in nutrition, and because the normal variation in output of spermatozoa was sufficiently large to obscure the subtle changes that might be expected to result from changes in the efficiency of spermiogenesis, for example.

The volume of semen collected altered within two weeks of the changes in the regimen of nutrition, perhaps reflecting changes in the level of testosterone secretion by the rams. The occurrence of an endocrine response within a week or so of the diets changing could reasonably have been expected following the observation that the mean ovulation rate of ewes may increase as early as six days after the onset of feeding lupins (Oldham and Lindsay 1984).

The output of spermatozoa from the rams of Group 1 was still increasing nine weeks after they had been placed on a high plane of nutrition and may have continued to increase until the rams reached maturity. In adult rams the output of spermatozoa probably continues to increase for at least nine weeks as testicular weight continued to increase for at least that long (Oldham et al. 1978). Therefore, from a practical point of view, feeding should begin at least nine weeks before the maximal output of spermatozoa is desired.

A difference in the daily production of spermatozoa per gram of testis contributed to the difference in the overall daily production of spermatozoa between rams of Groups 1 and 2 although the difference was not statistically significant. Daily production of spermatozoa per gram of testis in rams has also been shown to vary with nutrition (Oldham et al. 1978) and calculations based on the data of Hochereau-de Reviers et al. (1976) reveal that it also varies with season. In all of these experiments, daily production of spermatozoa per gram of testis was greatest after treatments that led to the greatest testicular weights. Thus, while measurement of testicular weight is a simple means of monitoring the effects of various treatments on the rate of production of spermatozoa, it should be recognized that it will tend to underestimate the extent of the changes in the rate of production of spermatozoa.

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