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Effect of Protein and Energy Content of the Diet on the Rate of Sperm Production in Rams

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Abstract

In the first experiment, the effects of two levels of dietary protein and energy on the number of sperm in the excurrent ducts, on the weight of the testes and on the weight of the seminal vesicles were examined in two-year-old Merino rams from two Peppin and two non-Peppin strains. There were large effects of diet on each of the three characters, the effect of energy intake being much greater than that of protein intake. In addition, there were significant differences between strains: Peppin strain rams had higher sperm numbers and testicular weights than non-Peppin strain rams, even though liveweights were similar.

In the second experiment, protein resistant to ruminal degradation was added to the basal ration to increase the quantity of amino acids absorbed from the intestines. Each of 10 rams was fed two diets in succession, and daily sperm production on each diet was estimated by the urine method. Daily sperm production was not affected by increasing the protein intake alone, but was significantly increased by increasing the intake of energy alone or of both protein and energy.

The results indicate that a high protein intake is not essential for high sperm production in rams; a level of 12 g crude protein digested in the intestines per 100 g digestible organic matter did not appear to be limiting for sperm production.

Introduction

Under Australian conditions, the diet of rams just before and during the joining period may often consist of pastures of advanced maturity that have a high fibre and low protein content. Thus, supplementary feeding may be necessary for maintenance of liveweight. However, there is a lack of information on the dietary levels of protein and energy needed to maintain a high level of sperm production in the ram.

Most earlier studies of the effect of level of nutrition on sperm production in domestic animals have used the number of sperm per ejaculate as a criterion (Moule 1963). However, this criterion is of very limited value unless a considerable number of ejaculates are taken over a short period—'exhaustive ejaculation' (Hale and Almquist 1960; Amann and Almquist 1962; Lino *et al.* 1967; Mattner and Braden 1967).

Salamon's (1964) results using this method indicated that the sperm production of Merino rams grazing natural pasture was significantly increased when the amount of both protein and energy supplied by supplementary feeding was increased. In the present studies, attempts were made to assess the relative importance of protein and energy intake on the rate of sperm production in adult rams.

Materials and Methods

When the present studies were initiated in 1962, the most suitable method available for estimating sperm production involved counting the number of sperm in the excurrent ducts (epididymis, vas deferens and ampulla). The results of the study using this technique suggested that the protein content

of the ration was of less importance than the energy content. However, in that study we could not be certain that the dietary intake of crude protein accurately reflected the amount of protein digested in the intestines. Therefore, when the technique of Ferguson *et al.* (1967) for prevention of ruminal degradation of dietary protein became available, the study was repeated. In the meantime, a better method for estimation of daily sperm production had been developed (Lino and Braden 1972). This method allows within-ram comparisons between successively applied treatments to be made, and thus avoids large inter-ram variation in sperm production, which was encountered in the first study.

(a) Experiment 1: Experimental Details

Sixty-two 2-year-old Merino rams, bred in the experiment reported by Dunlop (1962), were obtained from Armidale. There were 39 medium-wool Peppin strain rams (10 of strain A, 29 of strain B) and 23 non-Peppin rams [8 of strong-wool (S) and 15 of medium-wool (M) strains].

Table 1. Quantities of nutrients supplied per ram per week by the various rations in experiment 1

Ration	Dry matter (g)	Crude protein (g)	$10^{-3}\times$ gross energy (kcal)	$10^{-3}\times$ digestible energy (kcal)
Maintenance	3040	450	20·0	13·3
High energy–high protein (HE–HP)	4920	1005	32·0	23·6
High energy–low protein (HE–LP)	4180	245	23·3	18·7
Low energy–high protein (LE–HP)	2760	930	20·0	13·1
Low energy–low protein (LE–LP)	2380	240	16·4	10·7

The rams arrived at the Burdekin Unit, Glenfield Veterinary Research Station, N.S.W. Department of Agriculture, in May 1962. One month later they were shorn and their horns removed. The mean liveweight after shearing was 36·7 kg. Thereafter, the rams were weighed every 2 weeks and were fed a 'maintenance' ration consisting of 50 parts chopped wheaten hay, 37 parts maize and 13 parts linseed meal (see Table 1) for 15 weeks. At the conclusion of this period the mean liveweight was 37·1 kg. After placing the rams in order of liveweight, they were randomly allocated to six groups of eight rams and two groups of seven. Strain of ram was not taken into account in the randomization. Each of the eight groups was kept in a separate yard and randomly assigned to one of four rations which varied in energy and protein content (Table 1). The rations had the following composition:

HE–HP 35 parts wheaten hay, 34 parts maize, 31 parts linseed meal

HE–LP 80 parts maize, 15 parts maize starch, 5 parts wheaten straw

LE–HP 65 parts linseed meal, 35 parts wheaten straw

LE–LP 50 parts wheaten straw, 37 parts maize, 13 parts linseed meal

The crude protein levels were calculated from Kjeldahl nitrogen estimations; gross energy and digestible energy levels were calculated from published data. The rations were pelleted and supplied weekly in self-feeder bins.

After 11 weeks differential feeding, all of the rams were slaughtered by intracardiac injection of sodium pentobarbitone solution and the numbers of sperm in the excurrent ducts were estimated as described in the next paragraph. The weight of each testis and of the seminal vesicles was obtained soon after slaughter. The anterior pituitary glands were collected soon after slaughter of the rams and were placed in cold acetone; they were weighed after desiccation.

(b) Experiment 1: Estimation of Number of Sperm in the Excurrent Ducts

The abdomen was opened immediately after the injection of pentobarbitone and a ligature tied around the base of each ampulla to prevent post-mortem loss of spermatozoa into the urethra (Lino

1972). The testes, epididymides, vasa deferentia and ampullae were then dissected out. The vasa and ampullae were flushed from the proximal end using 25 ml of saline for each side (i.e. left and right). These washings were added to the fluid in which the epididymides were macerated. After the thick fibrous coat of the epididymis had been peeled off, the epididymis was cut into small segments, dropped into 200 ml of 0·9% saline and macerated in a mechanical macerator for 1–2 min at 14000 rev/min. The resulting suspension was strained through coarse muslin and two separate 1-ml samples of the fluid were taken. These were diluted appropriately and the sperm concentration estimated by use of haemocytometers. Sperm heads only were counted. Duplicate counts were made on each sample, left and right tracts being treated separately.

(*c*) *Experiment 2*

Ten mature Merino rams (five 3–4 years old, five 6–7 years old) were kept in single pens in an animal house and fed one of three experimental diets for 60–100 days before estimation of daily sperm production (DSP) by a 10–16-day urine collection. After this period they were fed another of the three diets and after 60–90 days the DSP was estimated in the same manner as before. Details of the method of DSP estimation are given by Lino and Braden (1972), with slight modifications (Braden *et al.*, unpublished data).

The basic ingredients of the diets used were: lucerne hay (40 parts), wheaten hay (10 parts), maize (30 parts), maize starch (20 parts) and minerals (sodium phosphate, manganese sulphate and sodium chloride). Three diets varying in protein content were prepared by adding 0, 5 or 10% of formaldehyde-treated casein to the basic ingredients. Each diet was ground and pelleted before use. The casein was treated dry in batches of 30 kg, 3 litres of water containing 300 g formaldehyde being sprayed onto each batch. Batches were then stored in sealed polythene bags before use (for details see experiment 4*a* of Hemsley *et al.* 1973). An *in vitro* digestion test indicated that the treated casein was resistant to ruminal degradation. A low energy–low protein (LE–LP) intake was achieved by feeding 700 g/day of the basic diet, a low energy–high protein (LE–HP) intake by feeding the ration containing 10% treated casein at 700 g/day and a high energy–high protein (HE–HP) intake by feeding 1050 g/day of the ration containing 5% casein. The diets are essentially the same as those used by Weston (1971) and, on the basis of his results, it would be expected that the LE–LP ration provided daily about 400 g digestible organic matter (DOM) and 48 g crude protein digested in the intestines (DCP_i). The corresponding values for the LE–HP ration would be 420 g DOM and 90 g DCP_i compared with 610 g DOM and 100 g DCP_i for the HE–HP ration; i.e. the DCP_i for the LE–LP, LE–HP and HE–HP diets was equivalent respectively to 12, 21·4 and 16·4 g per 100 g DOM intake.

Results

Experiment 1

It was not expected that there would be a significant effect of strain of ram on the number of sperm in the excurrent ducts, so that strain was ignored in allocating rams to treatment groups. However, when the rams were slaughtered at the end of the experiment, the Peppin strain rams were found to have significantly more sperm than non-Peppin rams, even though the liveweights of the two groups were similar. For both liveweight and pituitary gland dry weight there was a significant interaction between diet and strain of ram. Most of the contribution to this interaction for liveweight came from two exceptionally heavy S-strain rams on the HE–HP diet. The mean liveweights at slaughter of the rams on the four diets were: HE–HP, 48·7 kg; HE–LP, 43·6 kg; LE–HP, 39·0 kg; LE–LP, 35·4 kg. Corresponding pituitary gland weights were 169, 145, 130 and 110 mg respectively.

The estimated strain and diet means for the number of sperm in the excurrent ducts, weight of the testes and weight of the seminal vesicles are given in Table 2, together with the analyses of variance. The significance of the differences attributable to difference in energy intake, protein intake or strain of ram was tested using Scheffé's *S*-method (Scheffé 1959). The values of all these variables were significantly

greater ($P < 0{\cdot}01$ to $P < 0{\cdot}001$) in rams on the high-energy diets than in those on the low-energy diets (Table 3). The level of protein intake had no significant effect on seminal vesicle weight or on the number of sperm in the excurrent ducts. While it did produce a significant increase in the weight of the testes, the increase was considerably less than when energy intake was raised (Table 3). Rams of the Peppin strains (A and B) had significantly greater numbers of sperm in the excurrent ducts and significantly greater testes weights than rams of the non-Peppin strains (M and S).

Table 2. Estimated mean numbers of sperm and weights of testes and seminal vesicles for the rams in experiment 1

Diet or strain	$10^{-9}\times$ No. of sperm	Weight of testes (g)	Weight of seminal vesicles (g)
Diet:			
HE–HP	51·15	287·6	6·42
HE–LP	44·89	242·9	5·95
LE–HP	37·42	202·5	3·80
LE–LP	23·17	147·3	2·61
Strain:			
A	53·36	258·6	5·35
B	50·40	245·2	4·99
M	33·85	185·1	3·63
S	19·03	191·4	4·82

Summary of analyses of variance

Factor	D.F.	Liveweight M.S.	*F*	No. of sperm M.S.[A]	*F*	Weight of testes M.S.	*F*	Sem. ves. wt M.S.	*F*	Pituitary wt M.S.	*F*
Diet × strain	9	89·5	3·0**	1·36	0·4	3391	1·1	1·55	0·4	2626	2·4*
Diet	3	509·2		21·32	6·6***	52567	61·4***	48·09	14·2**	9376	
Strain	3	7·5		28·05	8·6***	18382	5·7**	7·80	2·3	1639	
Error 1[B]	46	29·9		3·62		3178		3·75		1105	
Error 2[C]	55			3·25		3213		3·39			

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$. *** $P < 0{\cdot}001$.

[A] $10^{-20}\times$ mean square.

[B] Error for testing interaction effects.

[C] Error for testing main effects in the absence of interactions.

Table 3. Differences in numbers of sperm and weights of testes and seminal vesicles attributable to levels of energy and protein intake and to strain of ram

Values given are ± standard error. Significance of differences was tested by the *S*-method of Scheffé (1959)

Factor	$10^{-9}\times$ No. of sperm	Weight of testes (g)	Weight of seminal vesicles (g)
Energy intake	31·96±9·2**	175·4±28·8***	6·01±0·94***
Protein intake	16·82±9·2	86·34±28·8*	1·43±0·94
Strain of ram	50·88±10·96***	127·3±32·37**	1·89±1·05

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$. *** $P < 0{\cdot}001$.

Experiment 2

An examination of the effect of increasing protein intake alone was made by comparing the effects of the LE–LP and LE–HP diets (rams 1, 2 and 3). The DSP values of the rams on the two diets (Table 4) were not significantly different. When both energy intake and protein intake were increased (comparison of the LE–LP and HE–HP diets; rams 4, 5 and 6), the DSP increased markedly (mean increase 71%). The increase was shown to be statistically significant ($P < 0{\cdot}01$) by a paired *t*-test. An examination of the effect of increasing energy intake alone (comparison of the LE–HP and HE–HP diets) was carried out with rams 7, 8, 9 and 10. The increase in DSP on the HE–HP diet was statistically significant ($P < 0{\cdot}02$), the mean increase being 26%.

Table 4. Effect of increasing the protein or energy intake or both on the daily sperm production (DSP) of rams in experiment 2

For details of diets and experimental procedure see Methods, section (*c*)

Comparison	Ram No.	Diet fed first	$10^{-9} \times$ DSP on: Diet A	Diet B	Percentage change
LE–LP (A) *v.* LE–HP (B)	1	LE–LP	5·98	6·20	+3·6
	2	LE–LP	7·57	9·02	+19·1
	3	LE–HP	5·77	4·77	−17·4
Means			6·44	6·66	+3·4
LE–LP (A) *v.* HE–HP (B)	4	LE–LP	2·48	4·42	+78·2
	5	HE–HP	3·75	6·33	+68·7
	6	HE–HP	2·96	4·96	+67·8
Means			3·06	5·24	+71·2
LE–HP (A) *v.* HE–HP (B)	7	LE–HP	5·59	7·59	+35·7
	8	LE–HP	4·37	5·83	+33·5
	9	HE–HP	5·11	6·27	+22·7
	10	HE–HP	5·37	6·08	+13·2
Means			5·11	6·44	+26·0

Discussion

The results indicate that diet has a marked influence on the rate of sperm production in rams. Increasing the energy intake of the ram had a greater effect on the DSP than did increasing the protein intake. Tilton *et al.* (1964) did not find a significant depression of sperm production in rams on a low protein intake or on a decreased energy intake (75% of control level). However, the criteria used (number of sperm per ejaculate or a 'depletion test' consisting of only eight ejaculates over 2 days) are unlikely to give a good indication of the DSP (Mattner and Braden 1967; Lino and Braden 1972).

In experiment 1, it was found that there was a significant difference between the rams on the high-protein and those on the low-protein diets in respect to weight of the testes. Since the protein in the diets was not deliberately protected against ruminal degradation, the high-protein diets possibly provided little more absorbed amino acid

than did the low-protein diets (Hogan and Weston 1967). However, the two high-protein diets apparently provided about 20% more digestible energy than their low-protein counterparts (Table 1), and thus the DCP_i/DOM ratios for the low-protein diets may not have been markedly lower than the ratios for the corresponding high-protein diets. In experiment 2, where the additional protein was protected from ruminal degradation by denaturation with formalin, there was no effect on the DSP when the DCP_i/DOM ratio was increased from 12 to 21. Thus it appears that, with diets providing more than about 12 g DCP_i/100 g DOM, protein is not the first nutrient limiting sperm production. For growth of young sheep, a comparable value is 18 g DCP_i/100 g DOM (Faichney 1971; Weston 1971). In experiment 2, when the daily energy intake was increased from 420 to 610 g DOM, the increase in DSP appeared to be lower than when both protein and energy were increased. The reason for this is not clear.

The energy content of the ration also appeared to affect the testosterone output, as indicated by the weight of the seminal vesicles. In a previous experiment (Moule *et al.* 1966), the fructose content of the ejaculate of rams was found to be reduced when the energy intake of the rams was reduced. Energy intake probably does not affect spermatogenesis or testosterone production directly, but may influence these characteristics via an effect on the output of gonadotrophins from the pituitary gland. In accord with this hypothesis is the observation that, in ewes, the ovulation rate is much more affected by the energy intake than by the protein intake (Memon *et al.* 1971; Braden *et al.*, unpublished data from an experiment using the same diets as those used in the present experiment 2).

Although the liveweights of Peppin and non-Peppin rams maintained on identical rations were similar, the weight of the testes and number of sperm in the excurrent ducts were significantly greater in the former than in the latter rams at the time of slaughter. This suggests that genetic effects on DSP and related characters may be significant and should not be entirely ignored. However, differences in sperm production between strains would be expected to have an observable effect on flock fertility only when the percentage of rams used in the flock is lower than usual, or when the environmental conditions (undernutrition, high ambient temperature, etc.) cause a markedly depressed DSP.

A change in the number of sperm available for ejaculation by the ram will not be apparent until at least 7 weeks after a nutritional change. Thus, in practical situations the nutrition of the ram in the 2 months prior to joining will influence the number of sperm available for impregnation of the ewes during the mating period. The present results suggest that diets high in protein are not essential for optimal sperm production; nor are they apparently able to increase ram libido (Mattner and Braden 1974).

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