STUDIES ON THE REPRODUCTIVE FAILURE OF RAM CAUSED BY UNDERFEEDING IV. ON THE TESTICULAR FUNCTION AND THE POTENCY OF ANTERIOR PITUITARY IN UNDERFED RAM

著者	MORI Akira, AMBO Kaichi, SUGAWARA Shichiro
journal or publication title	Tohoku journal of agricultural research
volume	10
number	4
page range	353-368
year	1960-01-20
URL	http://hdl.handle.net/10097/29292

STUDIES ON THE REPRODUCTIVE FAILURE OF RAM CAUSED BY UNDERFEEDING

IV. ON THE TESTICULAR FUNCTION AND THE POTENCY OF ANTERIOR PITUITARY IN UNDERFED RAM

By

Akira MORI, Kaichi AMBO, and Shichiro SUGAWARA

Department of Animal Husbandry, Faculty of Agriculture, Tohoku University, Sendai, Japan

(*Received September, 30, 1959*)

Introduction

Mori (8, 9, 10) has presented a series of reports on the reproductive difficulties in the rams caused by underfeeding. In those experiments, rams went into reproductive difficulties when they had been underfed, but when the impotent rams were rereared with normal foods or animal-protein-containing foods they recovered their mating potency. Injection of saline emulsion of anterior pituitary of sheep was also effective in a way for fighting the reproductive difficulties.

Whether this kind of reproductive difficulty is caused following the nutritional difficulty in the animal's physiological mechanisms by underfeeding or antecedes it, though the answer may be different according to the manner and the degree of underfeeding, seems to have been little studied.

The testicles have a function of spermatogenesis in the seminiferous tubules and at the same time produce male sex hormone in the interstitial cell. Both these functions of spermatogenesis and internal secretion are under the control of the gonadotrophin of the anterior pituitary gland. In the reproductive difficulty by underfeeding, the testicles grow degenerated (7) or malformed spermatozoa grow in number (4, 7, 9)—yet the functions of the testicles or the potence of male sex hormone in such cases seems to have been not yet cleared. Also, little studies seem to have been done on the potence of gonadotrophin of the anterior pituitary lobe.

The authors fed growing rams low-protein feed and confirmed the appearance of reproductive difficulties in their gonads, and then fed them normal feed. The purpose of this report is to observe the effectiveness of feeding normal feed upon their recovery. In this experiment, the others evaluated at the samer time the degree of their reproductive difficulties by the properties of their blood and their hepatic functions (1, 5, 14), and authors examined the function of the testicles, especially the potence of male sex hormone and of gonadotrophin of the anterior pituitary lobe.

We heartiy thank Profs. M. Umezu and S. Nishida who kindly guided us in accomplishing this study. We are also greatefull to Mr. Hayao Gonai, Miyagi Prefecture Sheep Association, who offered us rams, and to the Iwaya Poultry Breeding Station for placing newly hatched chickens at our service for animal assay.

Materials and Methods

Four heads of one year old ram of the Corriedale breed were used as the experimental animals.

During six weeks after the beginning of the experiment, the rams Nos. 1 and 2 were fed with normal feed (N) as control, while the rams Nos. 3 and 4 were fed with low protein feed (L, rice-plant straw). Six weeks later, the left testicles of all the rams were excised by the routine gelding method, and the testicles in the ram Nos. 3 and 4 were confirmed to have degenerated on account of underfeeding, the feeding schedule was changed.

The ram No. 3 was fed normal feed (N) to observe how the left testicle would recover. The other three were fed as before (No. 1–N, No. 2–N, No. 4–L) for 10 weeks thereafter (16 weeks since the beginning), then the rams Nos. 1 and 3 were fed with high protein feed (H).

The authors observed the properties of blood and the hepatic functions (upon BSP administration) in the periods from the beginning of the experiment till their death by slaughter, and at the slaughter the authors examined the weights of the chief organs, the functions of the testicles (histology, forms of spermatozoa, O_2 consumption, animal assay of male sex hormone) and animal assay of the gonadotrophin of the anterior pituitary gland.

Table 1 shows the details of the experimental rams and the feed given to them.

Table 1. Material rams and their feeding.

No. of ram	Groups	Date of birth	Age in days (19 Sept.)	Initial body wts. (19 Sept.)	Feeding			Date of slaughter
					-26 Sept.	27 Sept. -6, 7 Nov.	7 Nov.-	
1	N-N	1 April	172	16.7kg	N	N	H	27 Feb.
2	N-N	1 May	142	25.6	N	N	N	6 March
3	L-N	20 March	183	42.1	N	L	H	1 March
4	L-L	5 May	137	25.6	N	L	L	12 Feb.

Table 2. Kinds of feed and weights of digestible crude protein fed to a ram per kg.

No.	Groups	-26 Sept.	27 Sept.-6 Nov.		7 Nov.-17 Nov.		18 Nov.-	
		Feed	Feed	D.C.P.	Feed	D.C.P.	Feed	D.C.P.
1	N-N	Native grasses	Native grasses	—	Native grasses + Concent-rates	2.0g	Hay + Concent-rates	5.0g
2	N-N	〃	〃	—	〃 + 〃	2.0	〃 + 〃	2.0
3	N-L	〃	Rice plant straw	0.3g	〃 + 〃	2.0	〃 + 〃	5.0
4	L-L	〃	〃	0.3	Rice plant straw	0.3	Rice plant straw	0.3

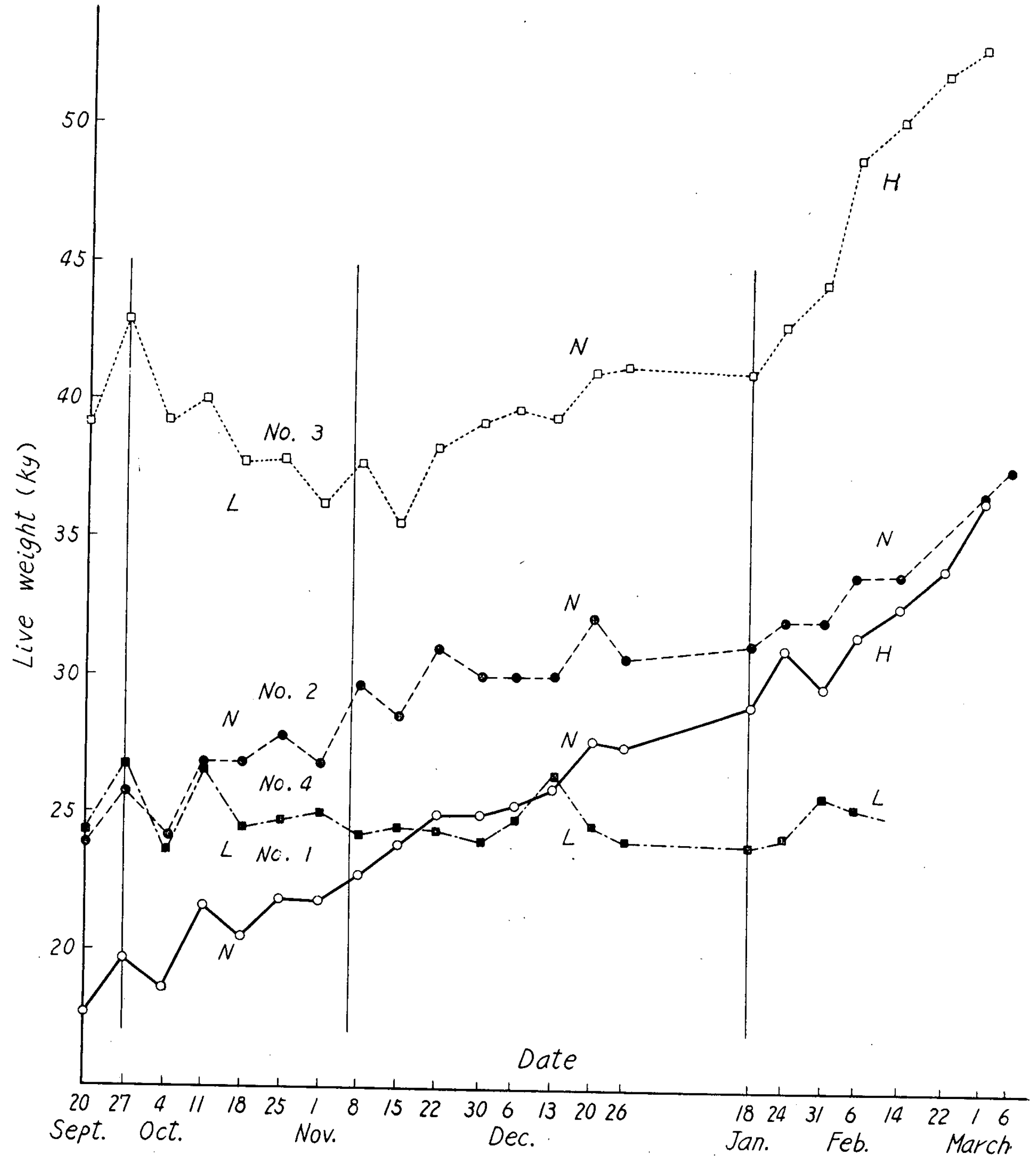

Fig. 1. Individual live-weight growth curves of rams on different planes of nutrition.

Table 2 shows the kinds of feed used and the weight of degestible crude protein fed to a ram per kg of body-weight a day.

Orchard grass was used for preparing hay, and soy-bean cakes and bran as concentrates.

Results

1. Changes in body-weight, weight of chief organs, properties of blood and hepatic functions

1) Changes in body-weight

The test, began on the 27th of September 1956, and continued till the time the rams were slaughtered. Fig. 1 shows the changes in their body-weight during the test periods.

The ram No. 1 (N–N–H). Gained weight, though not remarkably. In the last period, the H-feeding period, the gain became somewhat more perceptibly.

The ram No. 2 (N–N–N). Gained weight slightly.

The ram No. 3 (L–N–N). Lost weight in the L-feeding period, gained in the N-feeding period and gained further in the H-feeding period.

The ram No. 4 (L–L). Scarcely displayed changes in weight throughout the whole period.

2) Weight of the chief organs and meat production

The weights and their ratios to the body weight of the heart, the lungs, the liver, the kidneys, the spleen, the stomach and the pancreas are shown in Table 3, and meat production in Table 4.

Table 3. Weight of the chief organs and their ratios to body weight.

No.	Groups	Date of slaugher	Body wts. (kg)	Heart		Lungs		Liver		Kidneys		Spleen		Stomach		Pancreas	
				g	%	g	%	g	%	g	%	g	%	g	%	g	%
1	N-N	27 Feb.	36.5	145	.397	297	.764	608	1.665	87	.238	40	.109	700	1.918	52	.143
2	N-N	6 March	37.8	149	.394	271	.717	627	1.660	98	.260	44	.117	1050	2.778	56	.148
3	L-N	1 March	53.0	212	.400	447	.843	855	1.613	116	.219	58	.109	—	—	72	.136
4	L-L	12 Feb.	25.0	128	.512	261	1.044	261	1.044	52	.208	32	.128	200	.800	21	.084

Table 4. Meat production.

No.	Groups	Body wts. (kg)	Carcass wts. (kg)	Muscle wts. (kg)	Muscle wts. % body wts.
1	N-N	36.5	13.000	10.125	27.74
2	N-N	37.8	13.000	9.000	23.81
3	L-N	53.0	20.500	15.000	28.30
4	L-L	25.0	7.500	4.500	18.00

The per cent in body weight of the heart and the lungs was larger, but that of liver, the stomach and the spleen was remarkably smaller in the ram

No. 4 (L-L-L), than in the other three rams. The ram No. 3 (L-N-H) was fed with N or H from the influence of underfeeding.

As for the weight of the suprarenal glands of the rams Nos. 1-4, they were 0.03g, 0.03g, 0.05g, 0.06g in the order named, that of the ram No. 4 (L) being the heaviest.

A manifest influence of underfeeding can be observed in meat production (muscles) as shown in Table 4.

3) Properties of blood and hepatic function

The members of another group studying the nutritive difficulties observed the properties of blood and the hepatic function with the same miaterial

Table 5. Properties of blood and hepatic functions.

Items		No.	27 Sept.	6, 7 Nov.	18 Jan.	Final
Body weights (kg)		1	19.8	21.8	29.0	36.5
		2	25.8	26.8	31.2	37.5
		3	42.9	36.2	41.1	53.0
		4	26.7	25.0	23.9	25.0
Properties of blood	Serum protein (%)	1	5.0	5.2	5.3	5.1
		2	5.0	5.2	5.1	5.3
		3	5.2	4.6	5.2	5.4
		4	4.8	4.8	4.7	5.7
	Urea (mg/dl)	1	62	45	49	66
		2	62	52	56	66
		3	50	22	45	59
		4	55	17	29	29
	Volatile fatty acids (mg/dl)	1	4.8	7.2	7.2	5.4
		2	7.2	5.8	6.7	6.3
		3	7.0	3.4	9.2	7.1
		4	6.4	2.7	5.4	4.3
	Erythrocytes count (mm)	1	—	—	557	750
		2	—	—	480	550
		3	—	—	525	730
		4	—	—	449	350
	Hemoglobin content (g/100cc)	1	9.19	11.69	11.56	12.54
		2	9.59	10.53	9.93	10.21
		3	9.61	13.43	12.00	13.50
		4	10.15	10.67	8.60	9.35
Hepatic function	Liver's function of excreting foreign matter (tested by the BSP) (%)	1	15	31	50	47
		2	20	15	29	37
		3	26	78	45	64
		4	8	78	52	82
	Dehydrogenase function (mins.)	1	—	—	—	19′10″
		2	—	—	—	29′20″
		3	—	—	—	36′
		4	—	—	—	125′
	QO_2 (μl/mg/hr.)	1	—	—	—	1.50
		2	—	—	—	2.50
		3	—	—	—	1.90
		4	—	—	—	4.40

animals. The results of their observaton are shown in Table 5.

As seen in this table, the underfed rams show nutritional difficulties in the properties of blood such as the contents of serum protein, urea and volatile fatty acids, erythrocyte count and hemoglobin content, as well as in the dehydrogenase activity (Tünberg's method) and $Q0_2$ value of the liver. But the liver's function of excreting foreign matter (tested by the BSP clearance rate in blood), though remarkably lowered in the first week, recovered later.

The underfed rams used in this study showed such nutritional difficulties.

2. The testicles and their function

Here the weighe of the testicles and the epididymes, histological observation of the testicles, the forms of spermatozoa, O_2 consumption, dehydrogenase activity of the testicles, and chick assay of male sex hormone will be the subjects discussed.

1) Weights

The weights and the per cent in body weight of the testicles and epididymes are shown in Table 6.

Table 6. Weights and % body weight of testicles and epididymes.

		Testicles								
		Left				Right				
No.	Groups	Date	Body wts.	Testis wts.	% body wts.	Date	Body wts.	Testis wts.	% body wts.	Growth rate (duration)
1	N-N	6 Nov.	22.8kg	35.5g	0.156%	27 Feb.	36.5kg	97.5g	0.267%	174.65%(113)
2	N-N	7 Nov.	29.7	74.0	0.249	6 March	37.8	159.0	0.421	114.86 (120)
3	L-N	6 Nov.	37.7	29.0	0.077	1 March	53.0	104.0	0.196	258.62 (115)
4	L-L	7 Nov.	24.2	20.0	0.083	2 Feb.	25.0	76.0	0.304	280.00 (98)
		Epididymes								
1				8.5g*	0.037%**			19.0g*	0.056%**	123.53%***
2				20.0	0.069			20.8	0.055	4.00
3				8.5	0.023			20.0	0.038	135.29
4				4.0	0.016			8.5	0.034	112.50

Note: * means epididymes wts., ** % body wts. of epididymes and *** growth rate of epididymes.

The relative weight to the body weight of the testicles in the ram No. 3 (L) and the ram No. 4 (L) was smaller than those of the ram No.1 (N) and the ram No. 2 (N) at the time of castration But at the time of slaughter, the value in the No. 4 (L–L) was fairly high. These facts imply that the testicles can more or less grow under underfeeding of such a degree. Though the value is not high in the ram No. 3 (L–N), this smallness is considered to be simply due to the large relative increase in its body weight.

Also in the epididymes the effect of underfeeding upon their relative

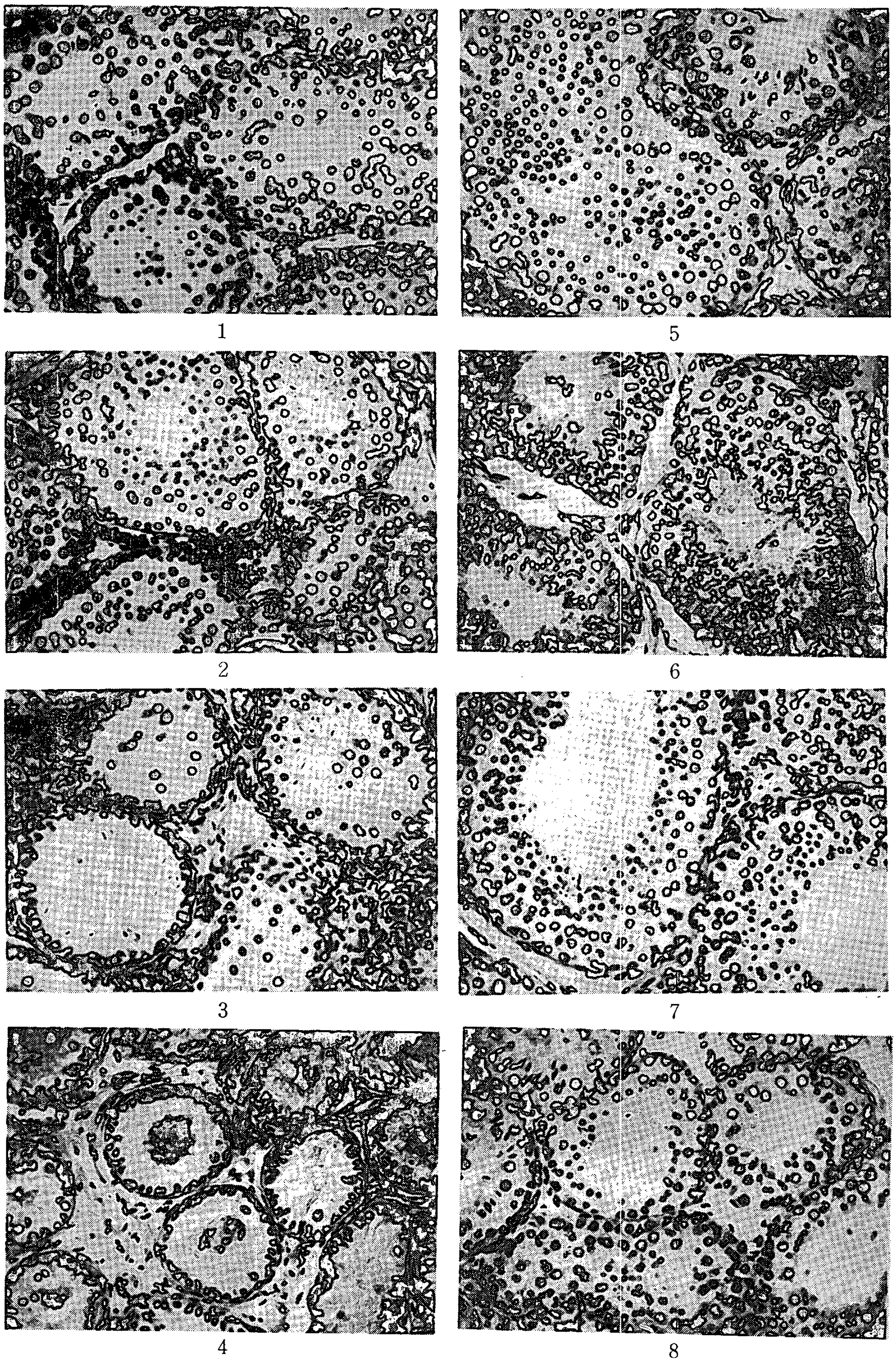

Fig. 2. Section of the testicles.

weight was observed at the castration. And they were apparently still small at the time of slaughter.

2) Histological observation of the testicles

The conditions of seminiferous tubules are shown in Table 7 and Fig. 2.

Table 7. Conditions of seminiferous tubules.

No.	Groups	Left: Seminiferous tubules	Left: Spermatozoa	Right: Seminiferous tubules	Right: Spermatozoa
1	N-N	Normal	+	Normal	+
2	N-N	Normal	+	Normal	+
3	L-N	Degeneration	+	Degeneration	+
4	L-L	Degeneration	−	Some deg.	+

The ram No. 1 (N–N) : The cells in the seminiferous tubules at the castration were normal (Fig. 2–1) and contained a few spermatozoa (Table 7). On the contrary, though the seminiferous tubules (Fig. 2–5) were normal also at the slaughter, their diameter was larger and more spermatozoa were contained then than at the castration.

The ram No. 2 (N–N) ; The tubules were normal both at the castration (Fig. 2–2) and at the slaughter (Fig. 2–6), and spermatozoa could be seen in both occasions. Their diameter was larger in the latter.

The ram No. 3 (L–N) : At the castration, the seminiferous tubules (Fig. 2–3) were remarkably degenerated—germ cells were few, and Sertoli cells occupied the bulk ; while at the time of slaughter (Fig. 2–7), they had become normal with larger diameter.

The ram No. 4 (L–L) : Remarkably degenerated. Sertoli cells occupied the whole, without any spermatozoa at the crstration (Fig. 2–4). At the slaughter (Fig. 2–8), they were normal, the diameter grown larger, and spermatozoa could be seen, in spite of the L-feed. Nevertheless the germ cells were obviously fewer in number than in the other rams (Fig. 2–5, 6, 7).

3) Forms of spermatozoa

Table 8 presents the forms and distribution (%) of malformed spermatozoa in the cauda epididymides at the castration (left) and at the slaughter (right) from February to March.

The ram No. 1 (N–N) : Spermatozoa existed in the testicles at the castration (Table 7), but the frequent lack of cauda epididymides may be due to their immaturity. The rate of malformation of spermatozoa was 22 per cent, which is rather high.

The ram No. 2 (N–N) : The rate of malformed spermatozoa was the lowest among the four rams.

The ram No. 3 (L–N) : The rate of malformed spermatozoa showed the

Table 8. From and distribution of malformed spermatozoa in the cauda epididymides.

No.	Groups		Percentage of malformed spermatozoa					
			Head	Neck	Mid-piece	Tail	Complex	Total
1	N-N	L						
		R	0	0.2	0.8	20.5	0.4	21.9
2	N-N	L	2.1	0.1	1.7	3.5	0.2	7.6
		R	0.1	0.1	0	5.9	0	6.1
3	L-N	L	1.5	0	0.4	44.0	4.5	50.4
		R	0.4	0.1	0.1	33.0	0.3	33.9
4	L-L	L						
		R	2.8	0.5	1.5	74.8	6.7	86.3

Notes: 1) L means left (at castration) and R means right (at slaughter).
2) No spermatozoon could be seen in the cauda epididymides of No. 1-L and No. 4-L.

high value of 50 per cent at the castration (Left, L-feed) but it was somewhat lowered at the slaughter (Right, N-feed).

The ram No. 4 (L–L): No spermatozoa at the castration (Left, L-feed), perhaps, on account of immaturity and L-feed. At the slaughter (Right, L-feed) spermatozoa exist, but the malformation rate of the spermatozoa showed a remarkably high value of 86 per cent.

In all the six cases in the above four rams, tail-malformation was a characteristically frequent form of malformed spermatozoa. Some malformed spermatozoa in the cauda epididymides at the time of castration (Left, L-feed) of the ram No. 3 are shown in Fig. 3.

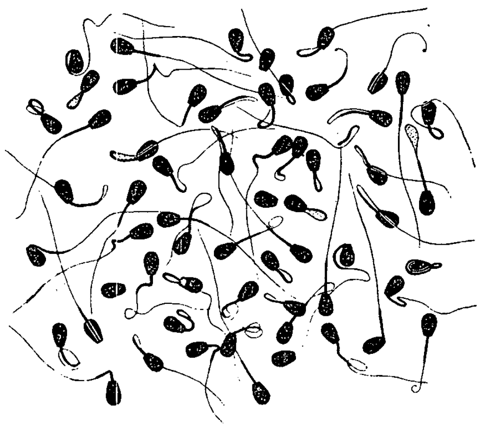

Fig. 3. Malformed spermatozoa (ram No. 3).

4) O_2 consumption in the testicles

The authors measured the O_2 consumption in the testicles (by Warburg's pressure meter) at castration (Left) and slaughter (Right). Glucose, propionate, butyrate, acetate and non-substrate were the base used for the purpose, but here the authors will mention the results with non-substrate alone. The results of the measurement are shown in Fig. 4.

At the castration time, the O_2 consumption of the underfed rams No. 3 and No. 4 was smaller than that of the rams normally fed (Nos. 1 and 2). But at the slaughter time, the value of the ram No. 4 became scarcely different from that of the other rams, and the smallest O_2 consumption was found in the ram No. 1. The low value of the ram No. 3 at the castration may as well be considered to be due to the lack of spermatozoa. However that may be, the O_2 consumption of the underfed ram No. 4 at the slaughter was, contrary to my anticipation, almost equal to or rather larger than that of the normally fed rams.

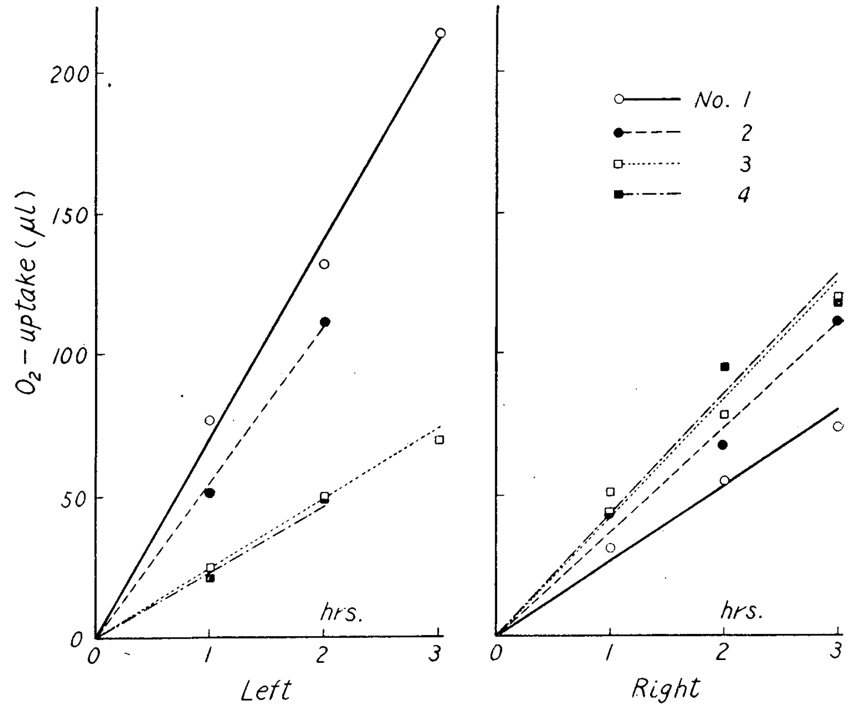

Fig. 4. Respiration of testes.

5) Animal assay af male sex hormone in the testicles

The authors sampled 29 g of the right testicular parenchyma from each ram at the slaughter, and made an extract from it by Gallagher & Koch's method (3), down to the step of acetone-extracting, diluted it with 10 cc of alcohol and some of it was diluted again to 1 : 4; using the two dilutions, the author tried bioassay on male chickens by comb reaction, with 5 γ and 1 γ of testosterone (in gross weight) as the control. Assay by alcohol application alone was also carried out. One application a day after hatch, ten times in total, of 0.005 cc each was given.

On the other hand, the authors also applied a suspension of testicular parenchyma mashed in equal weight of anhydro-alcohol.

The results of these tests are shown in Table 9.

As seen from Table 9, the results of assay by comb reaction with extract of testicular parenchyma shows that the relative weights of the combs of the chickens applied $\times 4$ dilution were not necessarliy lower than those of the chickens applied with the original dilution. This result is not entirely satisfactory. Nevertheless, the value of the chickens applied with the extract from No. 4 does not show conspicuous difference compared with the other three cases; moreover its value is higher than that of the control case C-3

(0.24) in which no testicular ingredient was used, and is almost equal to that of the case C-2. Therefore, the author is inclined to regard that the testicle of the ram No. 4 contains about 1 γ of male sex hormone. On the other hand,

Table 9. Comb-assay with extract and alcohol suspension of testicles.

No. of ram	Groups	Testicle extract: Dilution	Number of chickens	Body wts. g	Comb wts. mg	% body wts. %	Alcohol suspension: Dilution	Number of chickens	Body wts. g	Comb wts. mg	% body wts. %
1	N-N		5	54.4	30.6	0.056	—	5	68.5	58.4	0.085
		×4	5	61.8	45.6	0.074					
2	N-N		5	56.2	26.8	0.048	—	5	72.2	51.6	0.068
		×4	5	54.6	35.2	0.063					
3	L-N		5	65.4	49.0	0.075	—	5	67.3	63.0	0.093
		×4	3	61.7	35.0	0.059					
4	L-L		4	62.0	27.5	0.044	—	5	77.8	82.2	0.106
		×4	4	53.5	32.3	0.060					
		Testosteron					Testosteron				
Controls	1	5γ	5	53.4	82.2	0.0153	5γ	5	71.2	166.4	0.233
	2	1γ	5	50.6	39.6	0.0760	1γ	5	71.6	119.4	0.166
	3(12)	0	14	58.6	17.1	0.2400	0	5	74.6	43.6	0.058

the value shown by the chickens applied with alcohol suspension of testicular parenchyma of No. 4 was not lower than those of the other three.

3. Anterior pituitary gland and its function

The gonadotrophin (GTH) in the anterior pituitary gland acts upon the testicles to secrete follicle-stimulating hormone (FSH) and leuteinizing hormone (LH, ICSH).

To compare the potency of gonadotrophin in the anterior pituitary gland of the underfed ram (No. 4) with those of the other three rams under normal feed, the following bioassay was made.

1) Weight

Table 10 presents the weight of the anterior lobes of the pituitary gland (raw, dry, raw/dry, % body weight) sampled at the slaughter and the weight of their posterior lobes (raw). The dried specimens were prepared with acetone.

Table 10. Weights of pituitary gland.

No.	Groups	Body wts. kg	Anterior lobe: Raw mg	Dry mg	Dry/Raw %	% body wts.	Posterior lobe(raw) mg
1	N-N	36.5	479	117	24.43	14×10^{-6}	43
2	N-N	37.8	550	121	22.00	16×10^{-6}	71
3	L-N	53.0	442	98	22.17	10×10^{-6}	88
4	L-L	25.0	266	59	22.18	12×10^{-6}	35

In the ratio of the weight of the anterior pituitary lobes to the body weight, inferiority can be seen in the ram No. 3 (L-N) and the ram No. 4 (L-L). Yet, from these poor materials it cannot be judged whether the inferiority of the ram No. 3 was specially due to underfeeding.

2) Animal assay of the potency of gonadotrophin in the anterior pituitary gland on male chikens

The authors injected the gonadotrophin of the anterior pituitary lobe of the ram No. 1-4 into male chikens and examined the weight of the testicles biologically.

The anterior pituitary lobe was dehydrated no sooner than it was sampled, put into an evacuated glass tube for preservation and preserved in a refrigerator; it was powdered before use.

Chickens of White Leghorns were used. They were injected with powder-suspension of the anterior pituitary lobe four fimes every 24 hours, beginning at 12 hours after their hatch. They were sacrificed with ether 12 hours after the last injection, that is, 96 hours after the hatch, and their testicles were weighed.

Acetone powder of the anterior pituitary lobe was mingled with so much water, that is, one injection dose came up to 0.1 cc. The chickens were divided into five proups injected with 0.5, 1.0, 2.0, 4.0, 8.0 mg per head of the powder in total, respectively.

It was expected that the first three groups would be sufficient to obtain adequate data, but the results proved inadequate and the last two groups were newly added. The results are shown in Table 11.

Table 11. Chick-assay of potency of gonadotrophin in anterior pituitary.

Assay series		0.5 mg				1.0 mg				2.0 mg				4.0 mg				8.0 mg			
No.	Groups	N	B	T	%	N	B	T	%	N	B	T	%	N	B	T	%	N	B	T	%
1	N-N	4	24.6	6.3	.025	5	23.7	5.0	.021	5	24.6	5.7	.023	3	25.8	7.2	.028	3	24.7	7.3	.030
2	N-N	5	25.3	4.4	.017	5	24.4	5.6	.023	5	23.1	5.3	.023	3	24.7	9.2	.037	3	25.2	10.0	.040
3	L-N	5	26.7	5.3	.020	5	24.8	3.9	.015	5	25.2	5.4	.021	3	23.2	7.8	.034	3	24.5	9.8	.040
4	L-L	5	23.5	4.9	.021	5	23.8	5.9	.025	5	24.1	7.2	.030	3	24.5	11.2	.046	3	25.3	12.8	.051
Control		5	34.4	6.3	.019	5	24.1	5.5	.023					3	25.2	6.0	.024*				
Test of differences										4:1 0.2-0.1 4:2 0.1-0.05 4:3 0.3-0.2 4:C 0.05-0.02				4:1 0.1-0.05 4:C 0.05-0.02 4:C 0.2-0.1 2:C 0.4-0.3				4:1 0.01-0.001 4:2 0.1-0.05 4:3 0.05-0.02 4:C 0.01-0.001			

Notes: 1) N means number of chickens, B body weights, T testes weights and % ratio to body weights.
2) Control (C) were not injected.
3) The value marked * was used for determination of the difference from the control.

The effect of the gonadotrophin began to be visible in the 2.0 mg group only in the ram No. 4 (L–L), but the effectiveness was manifest in all the four rams of the 4.0 and 8.0 mg groups. The ratio of the weight of the lobe to the body weight was particularly large in the cases injected with suspension of powdered anterior lobe of the ram No. 4.

Discussion

One of the authors have already reported on the reproductive difficulties of male animals caused by feeding protein-free feed (7) or low-protein feed (9). In this study also, apparent reproductive difficulties were observed after six weeks of feeding extremely low-protein feed (rice-plant straw). That is to say, in the underfed rams, no increase in the body weight could be seen at all, diminution in weight of the testicles and their percentage in body weight, decrease of all germ cells in the seminiferous tubules, increase of malformed spermatozoa, and decline of O_2 consumption in the testicles were observed. The authors should like to consider that these phenomena were caused by underfeeding. Among the malformed spermatozoa, tail malformed spermatozoa highly increased and it was already known that malformed spermatozoa of the form appear particularly often under the influence of underfeeding (2, 4, 7, 9).

In their weight ratio to the body weight, in the underfed rams, the heart and the lung were the heaviest and the liver, the stomach and the pancreas were generally light. Though the cause cannot be explained readily, the effect of underfeeding must be the chief cause.

On the other hand, rather severe nutritional difficulties as well as reproductive difficulties begin to appear when rams are underfed. The difficulties are apparent especially in the function of the liver and the properties of blood.

It may depend on the degree of underfeeding, whether nutritional difficulty precedes reproductive difficulty or vice versa. In this study, the authors could not investigate into the relativity of reproductive difficulty with nutritional difficulty, being unable to observe the former day by day, on account of the small number of materials. The question would have been answered, however, to a certain extent, if microscopic observations of the seminiferous tubules through biopsy of the testicles, or observation of the forms and distribution of malformed spermatozoa were carried out.

The rams, in which reproductive and nutritional difficulties appeared as a result of underfeeding for six weeks, begin to recover when they are normally fed. Namely, they gain in weight, their organs become almost equal to the normally fed rams in percentage in body weight, the properties of their blood and their liver functions become normal, and they finally recover from nutritional difficulties. The quantity of muscles of the rams which were kept further underfed was poor enough, while that of the rams which were normally fed after the six weeks of underfeeding became rich.

Normal feeding was also effective for recovery from reproductive difficulties. The testicles did not gain in percentage body weight, but all the germ cells recovered from degeneration, malformed spermatozoa became fewer in number and O_2 consumption became almost equal to that of the rams fed normally. In the rams that were kept underfed following six weeks of underfeeding, the testicles grew to a certain degree and the spermatogenesis was still working, but malformed spermatozoa were still numerous. The O_2 consumption and the potency of male sex hormone of these rams, however, differed little from those of the normally fed rams.

Moreover, the potency of gonadotrophin in the anterior pituitary lobe was higher in the underfed rams than in the normally fed rams, which was surprising.

Here the authors came to believe that the decline of male's reproductive difficulty caused by underfeeding results, not from a diminished secretion of the gonadotrophin in the anterior pituitary gland, but from the incapability of maintaining the reproductive function owing to the nutritional difficulties, especially, a shortage in amino acid, in spite of more than normal or at least normal secretion of gonadotrophin.

Conclusion

Of the three kinds of rams that were fed with low-protein feed, that were fed with normal feed, and that were at first fed with low-protein feed and then with normal feed, the authors observed, measured and examined the changes in body weight, the weight of the chief organs, the properties of blood and the liver functions, and especially the weight of the testicles and the epididymes, the conditions of the seminiferous tubules, the existence of spermatozoa and the rate of their malformation, O_2 consumption and dehydrogenase activity of the testicles, the potency of male sex hormone in the testicles, the weight of the pituitary gland and the potency of the gonadotrophin in the anterior pituitary lobe. As the results of being fed with low-protein feed, these rams fell in nutritional difficulty, losing their body-weight; their plasma-protein, urea, volatile fatty acids, erythrocyte and hemoglobin in blood decreasing in number or in quantity, and their dehydrogenase activity and RQ value of liver diminishing.

However, though remarkable disturbances could be seen in the functions such as growth, spermatogenesis, respiratory capacity and dehydrogenase activity, in the potency of male hormone of the testicles, and also in the potency of the gonadotrophin of the pituitary gland as they were fed with low-protein feed, yet on the other hand, cases of unexpected functional normally were observed. The evident anomalies caused by low-protein feed are, first, a disturbance in spermatogenesis seen in the high increase of malformed spermatozoa, and second, a rise of potency of the gonadotrophin in the anterior pituitary gland. A ram which has a difficulty in the functions of the testicles caused by low-protein feed recover from it gradually when subsequently fed with normal feed.

As for the correlation between nutritional difficulties and reproductive difficulties which appear in an animal's physiological mechanisms when it is fed with low-protein feed, and also as for the correlativity between the pituitary gland and the gonad-function of the male animals fed with low-nutritional feed, the authors espect to investigate in a later study as an important problem.

Summary

Using four rams of the Corriedale breed one year old (numbered 1–4), the authors subjected the rams Nos. 1 and 2 to normal feeding, and the rams Nos. 3 and 4 to nuderfeeding (rice-plant straw). The left testicles and the epididymes of these rams were taken out by castration. Degeneration of the testicles of the ram Nos. 3 and No. 4 by underfeeding being ascertained, the No. 3 alone was normally fed to observe its state of recovery. The rest were fed as before, and all were slaughtered 20 to 23 weeks after the beginning of the experiment.

Besides the properties of blood, the liver function and the weight of the chief organs, the functions of the testicles and the anterior lobe of the pituitary gland were observed.

The results were as follows:

1) The body-weight was increased by normal feeding, and decreased by underfeeding.

2) The percent in body weight of the heart and the lungs was heavier in the underfed rams, and that of the liver, the stomach and the pancreas was heavier in the normally fed rams.

3) The total weight of the muscles of the underfed rams was lower than that of the normally fed rams.

4) Symptons of nutritional difficulties observed in the properties of blood, such as the contents of plasmaprotein, urea, volatile fatty acids, the erythrocyte count, the hemoglobin content, and also in the dehydrogenase activity and the RQ values of liver, were more remarkadly apparent in the underfed rams than in the normally fed rams.

5) The percent in body weight of the testicles and the epididymes were lowered by the six week's underfeeding, but were not particularly lowered by underfeeding of several weeks more.

6) The seminiferous tubules degenerated by being underfed, and recovered

when normally fed.

7) The rate of malformed spermatozoa in the cauda epididymides of the underfed ram was higher in the normally fed rams. The rate dropped by normal feeding. The rate of the malformed spermatozoa was extremely high in the continuously underfed ram. Most of the malformed spermatozoa were malfomed in their tails.

8) At the time of castration, O_2 consumption of the testicles was lower in the underfed rams, yet no influence of underfeeding could be seen at the time of slaughter.

9) The potency of male sex hormone in the testicles at the castration was found, through animal assay, to be lower in the underfed rams than in the normally fed rams.

10) The potency of gonadotrophin in the anterior pituitary lobe was rather higher in the underfed rams than in the normally fed rams.

Literature

1) Ambo, K. et al. Unpublished data.
2) Cunningham, I. J., and C. S. M. Hopkirk (1935). N. Z. J. Sci. Tech., **17**, 420. (Anderson, J. 1945).
3) Gallagher, T. F., and V. F. C. Koch (1929). J. Biol. Chem., **84**, 498.
4) Habu, Y., and S. Masuda (1936). Annual Report of Cyugoku Society of Japanese Native Cattle, No. 3. (in Japanese).
5) Kimura, K. Unpublished data.
6) Masuda, S., and H. Hashimoto (1956). Jap. J. Zootech. Sci., **8**, 119. (in Japanese).
7) Mori, A. (1952). Tohoku J. Agr. Res, **2**, 1.
8) Mori, A. (1957). Jap. Soc. Anim. Rep., **2**, 98. (in Japanese).
9) Mori, A. (1959a). Tohoku J. Agr. Res., **10**, 263.
10) Mori, A. (1959b). Tohoku J. Agr. Res., **10**, 283.
11) Mori, A. (1959c). Tohoku J. Agr. Res., **10**. 347.
12) Ouchi, T. (1956). Graduation Thesis of the Master of Arts Course, Agricultural Institute, Tohoku University. (in Japanese).
13) Severinghaus, A. E. (1939). Sex and Internal Secretion.
14) Sugawara, S. et al. Unpublished data.