The Effect of High- and Low-plane Feeding on the Serum Protein Levels of Pregnant Ewes, Foetuses and Young Lambs

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This communication presents some results obtained in a large-scale investigation of the effect of different dietary levels on the health and wellbeing of pregnant ewes and their lambs. Other aspects have already been described elsewhere (Thomson & Thomson, 1949). The data recorded here are the serum protein levels of the blood samples of pregnant ewes and their foetuses at approximately 10 days before term, and of lambs at varying brief intervals after birth. Certain figures obtained from blood samples of pregnant sheep and foetuses not included in the nutritional experiment are recorded with a view to supplying as much data as possible on such sera.

EXPERIMENTAL

Management of animals

The sheep used in the nutritional experiment were half-bred ewes crossed with a Suffolk tup and were about 1 yr. old at tupping. These animals were tupped while at grass and were only brought indoors for controlled feeding at about the sixth week in pregnancy. From then on they were divided into two groups, one being fed on a high plane and the other on a low plane of nutrition. Both groups received the same amounts of chop (oat straw and hay) and cod liver oil, but the diets differed both in energy and protein content due to differences in the amounts of roots and meals mixture fed. These diets are described in Table 1.

Table 1. *Daily rations of 'high'- and 'low'-plane ewes*

	High plane	Low plane
'Chop' (lb.)	0.5	0.5
Meals (lb.)	1.4 up to 2.0	0.75 down to 0.6
Roots (lb.)	4.2 up to 5.1	2.25 down to 1.8
Cod liver oil (ml.)	5	5
Gross digestible energy (lb.)	1.67 to 2.20	1.02 to 0.82
Protein equivalent (lb.)	0.19 to 0.25	0.11 to 0.08

The meals mixture consisted of ground maize, 4 parts; ground oats, 1 part; linseed cake meal, 4 parts; ground chalk, 2% (w/w); salt, 1% (w/w). The feeding was designed so that, by varying the meals intake over the pregnancy period from the sixth week to term, the high-plane ewes should gain about 40–50 lb. in weight, whilst the low-plane ewes should about maintain their weight. Actually the animals referred to in this communication put on an average of 47 lb. in the high-plane group and 7.5 lb. in the low-plane group. It should be noted that the small increase in the low-plane group and the large increase in the high-plane group is, in

part, due to the fact that the ewes were immature and still growing.

Operative technique. At approximately the tenth day before the calculated date of parturition the individual ewes were submitted, under chloral hydrate anaesthesia, to Caesarean section and, whilst still alive and before the foetus was removed from the uterus, samples of foetal blood were obtained from the umbilical vein and samples of maternal blood from the jugular vein. In the case of twins, samples were obtained separately from each foetus.

Analytical methods

Total serum protein concentration. This was measured by determination of total N by a micro-Kjeldahl method, using SeO_2 as a catalyst and employing a Markham distillation apparatus.

Albumin total protein ratios. The globulins were precipitated by saturating with MgSO_4 : to 1 ml. of serum were added 1.2 g. of finely powdered MgSO_4 and 7 ml. of a saturated aqueous solution of MgSO_4 . The mixture, with occasional shaking, was left at room temperature for 24 hr., after which it was filtered through a Whatman no. 42 filter paper and the albumin N was determined on a sample of the crystal-clear filtrate.

Electrophoresis. The serum samples were dialysed against phosphate buffer, pH 8, $I=0.2$ at 0° . In one case a veronal buffer (Michaelis, 1930) of pH 8.6 was used. The difference in refraction between the inner fluid (n_i) and the outer (n_o) was measured with a dipping refractometer. The dialysed sera were examined in a Tiselius (1937) apparatus, optical observations being made with the diagonal schlieren method and monochromatic light (546 m μ). The relative concentration of the components was measured by optical projection, tracing of photographs, extrapolation and planimetry. Anode and cathode limb patterns were used wherever possible, and in many cases two or more patterns from each limb obtained at different points of the run were analysed. Discrepancies in the results obtained from both limbs of the U-tube are expressed in subsequent tables as the range of error, although this may actually be greater (Svensson, 1946). In some cases where light dispersion due to lipid was marked, e.g. lamb and sheep sera, the sera were defatted by the method of McFarlane (1942). The double-length section cell described by Longworth, Cannon & MacInnes (1940) was frequently employed as the sheep globulins showed poor separation.

RESULTS AND DISCUSSION

The quantitative findings for the two experimental groups of animals are shown in Table 2. It will be seen that the blood sera of the ewes in the high-plane

Table 2. *Protein content of sera of ewes and foetuses (10 days before parturition) in relation to plane of nutrition*

Ewe no.	Ewe			Foetus		
	Total protein (g./100 ml.)	Albumin/total protein as percentage determined by		Total protein (g./100 ml.)	Albumin/total protein as percentage determined by	
		MgSO ₄ ppt.	Electrophoresis		MgSO ₄ ppt.	Electrophoresis
			High-plane ewes			
11	4.93	—		4.30	—	—
44	4.75	74.3	—	4.01	90.5	—
76	4.98	66.2	—	{ <i>F</i> ₁ 4.12*	98.0	—
71	4.40	68.1	—	{ <i>F</i> ₂ 3.94	98.0	—
36	5.63	65.0	—	3.36	92.4	—
33	6.09	61.1	—	4.47	78.3	—
39	4.94	73.5	67.3†	4.39	85.4	73.0 ± 2
				4.13	84.3	69.2†
Mean	5.07	68.0	—	4.09	89.6	—
S.D.	0.54	—	—	0.34	—	—
			Low-plane ewes			
43	4.75	—	—	4.24	—	—
40	4.31	65.4	—	3.70	87.6	—
23	3.96	66.1	—	{ <i>F</i> ₁ 3.46	88.4	—
32	4.65	70.1	—	{ <i>F</i> ₂ 3.38	84.0	—
55	4.12	66.7	—	3.75	86.9	—
49	4.69	72.0	62.6 ± 0	3.52	76.1	—
				3.94	83.2	75.0 ± 3
69	4.04	71.0	—	{ <i>F</i> ₁ 2.71	100.0	65.0 ± 4
				{ <i>F</i> ₂ 3.01	84.7	77.0†
Mean	4.36	68.6	—	3.52	86.4	—
S.D.	0.34	—	—	0.46	—	—
<i>t</i>	2.93	—	—	2.85	—	—

* *F*₁ and *F*₂ refer to twin lambs.

† Analysis of cathode side only.

‡ Analysis of anode side only.

Table 3. *Lamb sera analyses in relation to plane of nutrition of their dams*

Ewe no.	Lamb no.	Total protein (g./100 ml.)	Albumin/total protein as percentage determined by		Age of lamb (days)
			MgSO ₄ ppt.	Electrophoresis	
Lambs from high-plane ewes					
42	110	6.62	38.7	32.5±1	1
30	93	5.86	53.2	47.3±3	4
52	100	5.92	55.2	43.9±1	4
51	97	5.16	69.8	55.5±2	10
25	106	6.04	64.6	51.8±1	10
	Mean	5.92	56.2	—	—
	S.D.	0.52	—	—	—
Lambs from low-plane ewes					
28	102	4.12	70.9	65.0±4	1
53	105	4.74	62.0	38.4±1	1
22	82	5.96	47.0	—	4
34	87	5.10	54.9	—	4
26	88	4.24	76.4	62.6	10
37	92	4.08	—	—	10
	Mean	4.71	62.2	—	—
	S.D.	0.73	—	—	—
	<i>t</i>	3.10	—	—	—

group had a higher total protein content than the sera of the low-plane ewes. This difference was statistically significant, the '*t*' value being 2.93. The mean value for albumin nitrogen expressed as a percentage of total nitrogen showed no significant difference between the groups. Similarly, for the

sera of the foetuses, those from the high-plane group were significantly higher in total protein than those from the low-plane group, the '*t*' value being 2.85. The slight difference between the mean albumin/total protein ratios of the two groups was not significant. The few comparisons possible between the results

obtained for the albumin/total protein ratios, as obtained by the magnesium sulphate precipitation method and by electrophoresis, show, in every case, a lower result by the second method. Such differences are known to occur on account of solubility char-

acteristics of the proteins and they are possibly accentuated in this instance, owing to the adoption of unfavourable conditions such as degree of dilution and buffer ionic strength in the electrophoretic analyses of the sera of this particular species.

Table 3 shows the results for samples of serum obtained from lambs of the two groups at different

intervals up to 10 days after birth. All the lambs had been allowed to suckle normally in the interval. Here again the mean value, 5.92 g./100 ml., for the total protein of the sera of the lambs from the high-plane ewes was significantly higher than the mean value, 4.71 g./100 ml., for the sera of the lambs from the low-plane ewes, the 't' value being 3.10. The mean values for the albumin/total protein ratio show a slight but not significant difference between the two groups. For each group they are, however, lower than the corresponding values for the sera from either ewes or foetuses from the same groups. Since,

Fig. 1. Electrophoresis patterns of adult and foetal sheep sera. *a*, normal sheep serum and method of extrapolation. *b*, serum of foetus about 10 days before term from low plane-fed ewe. *c*, serum of 112-day foetus from sheep on pasture. *d*, serum of ram on pasture.

however, a globulin fraction appears in the serum of newborn lambs on suckling, as has recently been shown by Charlwood & Thomson (1948), this might

In our experiments, electrophoretic analyses of sera from sheep at different stages of pregnancy, from foetuses and from newborn lambs 1, 4 and

Fig. 2. Electrophoresis patterns of lamb and sheep sera. *a*, serum of unsuckled lamb of pasture-fed ewe. *b* and *c*, sera from two 1-day-old lambs from different low-plane fed mothers. The method of extrapolation is shown. *d*, serum of 4-day-old lamb from high-plane mother. *e* and *f*, serum of two 10-day-old lambs from high-plane mothers; *f*, after defatting. *g*, normal, and *h*, defatted serum of ewe at 112 days pregnancy.

be expected. These authors published electrophoretic patterns of sera from sheep and from their lambs before and after suckling, but did not give any quantitative data.

10 days old were made. Fig. 1*a* shows a typical electrophoretic pattern of ewe serum. As efforts to resolve all the components by extrapolation were unsuccessful the method of analysis outlined was

used. Only the albumin and the slowest peak component 2 were measured and the intermediate part of the pattern, obviously not homogeneous, was termed for convenience component 1. Although better separation was effected with veronal buffer, the indistinctness of the picture obtained, and the introduction of the double length section cell facilitating prolonged runs with the phosphate buffer, did not appear to justify the further use of the more expensive salt.

obtained from a foetus (no. 49) is shown in Fig. 1*b*. The serum was examined about 10 days before term. Only two main components are present, albumin and component 1, which latter must be identical with, or partly composed of, fetuin, the low molecular weight globulin described by Pedersen (1944). The method of extrapolation is obvious from the diagram. Fig. 1*c* presents the pattern of a foetal serum from a Welsh mountain ewe. This serum contains a well-marked component designated as component 2.

Table 4. *Electrophoretic analyses of sheep sera*

Source	Days in lamb	As percentages of total protein			Buffer	<i>I</i>	pH	Duration of electrophoresis (sec.)	Difference in refraction* ($n_1 - n_0$)
		Albumin	Component 1	Component 2					
Ram	—	69.8±2	18.0±1	12.2±2	Phosphate	0.2	8.0	14,520	0.00364
Ewe	NP†	36.7±4	24.1±5	39.2±8	Veronal	0.1	8.6	9,120	0.00314
Ewe	58	41.1±2	22.8±3	36.1±2	Phosphate	0.2	8.0	23,100	0.00320
Ewe	58	40.8±2	32.6±3	26.6±1	Phosphate	0.2	8.0	15,920	0.00323
Ewe	98	43.4±4	22.0±2	34.5±2	Phosphate	0.2	8.0	13,920	0.00314
Ewe	112	44.3±0	19.4±3	36.3±3	Phosphate	0.2	8.0	14,460	0.00300
Ewe	112	46.7±2	21.8±3	31.5±2	Phosphate	0.2	8.0	17,160	0.00396
(defatted)									
Ewe	114	59.1±2	23.0±1	18.0±2	Phosphate	0.2	8.0	21,000	—
Ewe	125	44.5±2	27.6±2	22.8±1	Phosphate	0.2	8.0	16,000	—
Ewe	132	53.6±3	26.2±1	20.2±3	Phosphate	0.2	8.0	30,000	0.00633
Ewe†	135-138	67.3	21.4	11.3	Phosphate	0.2	8.0	8,820	0.00300§
Ewe†	137-139	62.6	26.5	10.9	Phosphate	0.2	8.0	13,200	0.00327§
Ewe	146	57.0±2	21.4±2	21.6±1	Phosphate	0.2	8.0	24,000	0.00308

* Dialysed sera: n_1 , refraction of inner fluid; n_0 , of outer.

† NP means not pregnant.

‡ Stall-fed ewes. The other animals were on pasture.

§ Analysis of cathode side only.

Table 5. *Electrophoretic analyses of foetal sera*

(All the samples were dialysed against phosphate buffer of pH 8 and *I* 0.2.)

Age of foetus (days)	As percentages of total protein			Duration of electrophoresis (sec.)	Difference in refraction† ($n_1 - n_0$)
	Albumin	Component 1	Component 2		
98*	65.0	23.6	11.4	13,620	0.00317
112*	66.2±3	24.9±5	8.9±1	16,200	0.00414
135	73.0±2	27.0±2	—	13,680	0.00280
135	65.0±4	35.0±4	—	22,300	0.00170
135	77.0	23.0	—	14,220	0.00296
136	69.2	30.8	5.0 (trace?)	30,060	0.00404
134	75.0±3	75.0±3	—	13,440	0.00316

* The mothers (Welsh ewes) of these animals were on pasture; the remainder were stall fed.

† Dialysed sera: n_1 , refraction of inner fluid; n_0 , of outer.

In Table 4 are presented the results of the electrophoretic analyses of 12 sheep sera from ewes at different stages of pregnancy and one from a ram (Fig. 1*e*). Component 2, expressed as a percentage of the total protein, appears to fall towards the end of pregnancy and there is a corresponding rise in albumin. Further data on this point, preferably from the sera of ewes examined continuously through pregnancy, are desirable. The apparent fall in component 2 at the later stage of pregnancy suggests an association with colostrum formation.

A typical foetal serum electrophoretic pattern

A similar result was obtained from another foetus from a different mother of the same breed.

Table 5 gives the results of the electrophoretic analyses of a number of foetal sera from ewes of two breeds which were on different systems and planes of nutrition. Evidence of the presence of component 2 was found only in the sera from three of the foetuses.

Fig. 2*a* shows the electrophoretic pattern of the serum from a newborn lamb before suckling. This lamb was born from a Welsh ewe and component 2 is present. The patterns for the sera from two 1-day-old lambs are shown in Fig. 2*b, c*. Only in the second

Table 6. *Electrophoretic analyses of lamb sera*

Ewe no.	Lamb no.	Age at sampling	Percentages of total protein			Duration of electrophoresis (sec.)	$(n_1 - n_0)$
			Albumin	Component 1	Component 2		
—	757*	15–30 min.	69.0	28.5	2.5	18,960	0.00293
28	102	1 day	65.0±4	25.1±4	9.9±2	10,560	0.00362
53	105	1 "	38.4±1	26.1±12	25.5±1	23,000	0.00297
42	110	4 days	32.5±1	23.6	43.9	13,560	0.00350
30	93	4 "	47.3±3	22.1±4	30.5±2	14,000	0.00354
Ditto defatted		4 "	41.6±3	26.1±1	32.2±1	99,420	0.00227
52	100	4 "	43.9±1	28.4±1	27.6±1	21,840	0.00300
26	88	10 "	62.6	37.4		13,560	0.00280
Ditto defatted		10 "	60.0±2		11.9±2	13,440	0.00280
25	106	10 "	51.8±1		23.7±0	18,000	—
Ditto defatted		10 "	53.5	24.8±2	21.7±2	11,640	0.00356
51	97	10 "	53.8±1	27.1±1	19.1±1	13,260	0.00300
Ditto defatted		10 "	55.5±2	28.3±3	16.1±1	11,640	0.00296

* This lamb had not suckled. All others had suckled normally. Number of feeds about 6–8 for 1-day-old lambs (equal to 500–600 ml. of colostrum or milk).

is component 2 well marked, although it is presumed that suckling had occurred in both cases. Fig. 2*d* is the pattern for a 4-day-old lamb and Fig. 2*e, f* for 10-day-old lambs, in each case from a high-plane ewe. Whilst component 2 is well marked in Fig. 2*d*, it is much reduced in the two latter. There is also an anomaly in the latter which is strikingly similar with that described by Longworth, Shedlovsky & MacInnes (1939) and which, like this, disappears on delipidation (Fig. 2*f*). Fig. 2*g* shows this anomaly also present in the serum of an adult sheep, again disappearing on delipidation, although it is less marked than in the case of the serum of the 10-day-old lamb.

Table 6 gives the details of the electrophoretic analyses of the sera of a series of lambs of different ages. Except for the first lamb they would all have suckled normally. The number of feeds for a day-old lamb would probably be six to eight and equivalent to 500–600 ml. of milk or colostrum.

SUMMARY

1. Results are presented which show that, in the case of pregnant ewes on a high plane of nutrition, their blood sera, obtained approximately 10 days before term, had a higher total protein content than that of ewes on a low plane of nutrition. The same was true of the respective foetal blood sera obtained at the same time. There was no significant difference between the albumin/total protein ratios of the two sets of sera.

2. The same findings hold good for the blood sera of newborn lambs up to 10 days of age, from the ewes fed at high- and low-plane levels respectively.

3. The detailed results of the electrophoretic analysis of some of these sera, as well as those of other ewes, fetuses and lambs, are presented. There was evidence of a fall in component 2 in the sera from the ewes in the latter stage of pregnancy. It is suggested that this may be associated with colostrum formation.

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