Br. J. Nutr. (1967), **21**, 879

A study of the protein requirements of the mature breeding ewe

2.* Protein utilization in the pregnant ewe

BY J. J. ROBINSON AND T. J. FORBES

Agricultural Research Institute of Northern Ireland, Hillsborough and The Queen's University of Belfast

(*Received* 5 *April* 1967—*Accepted* 3 *July* 1967)

1. An experiment was carried out in which protein utilization in the pregnant ewe was studied using the nitrogen balance technique.

2. Eight diets supplying four different intakes of crude protein and two different intakes of energy were each offered to eight individually penned ewes.

3. The mean crude protein intakes per day were 7·2, 5·5, 4·1 and 3·0 g/kg $W^{0.73}$ (where W = body-weight) and the metabolizable energy intakes 134 and 113 kcal/kg $W^{0.73}$.

4. N balances were carried out at 10–12, 14–16 and 18–20 weeks of gestation on five ewes from each treatment.

5. The apparent digestibility of both dry matter and crude protein decreased with decreasing protein intake. With the high energy intake, the apparent dry-matter digestibility was increased and the apparent digestibility of crude protein decreased. Stage of gestation had no significant effect on the apparent digestibility of either of these constituents.

6. N retention was not affected by the number of foetuses carried. With the higher energy intake and the higher protein intakes, the absolute retention of N was significantly increased at all stages of gestation. N retention increased with advancing pregnancy; the retentions at 10–12, 14–16 and 18–20 weeks of gestation being 0·086, 0·114 and 0·163 g/kg $W^{0.73}$ per day respectively.

7. The efficiency of utilization of apparently digested N was calculated from the regression of retained N as a percentage of apparently digested N against apparently digested N.

8. The daily intakes of apparently digested N required for maximum efficiency were 0·551 and 0·620 g/kg $W^{0.73}$ on the high and low energy intakes respectively. The daily intake for maximum efficiency decreased with advancing pregnancy, the values being 0·623, 0·587 and 0·567 g/kg $W^{0.73}$ for the 10–12, 14–16 and 18–20 weeks of gestation respectively.

9. The levels of N retained at maximum efficiency were 0·235 and 0·202 g/kg $W^{0.73}$ per day for the high and low energy intakes respectively. The levels of N retained increased during pregnancy from 0·170 g/kg $W^{0.73}$ per day at 10–12 weeks to 0·286 g/kg $W^{0.73}$ at 18–20 weeks. The requirements for zero N balance were 0·072 and 0·153 g apparently digested N/kg $W^{0.73}$ per day for the high and low energy intakes respectively. The requirement for zero N balance decreased from 0·176 g/kg $W^{0.73}$ per day at 10–12 weeks to 0·071 g/kg $W^{0.73}$ at 18–20 weeks.

10. The results are discussed in relation to other research findings and current recommendations.

A daily intake of approximately 120 g digestible crude protein during the last 6 weeks of gestation has been widely accepted as the standard protein requirement of the mature (68 kg) breeding ewe (Thomson & Aitken, 1959; Morrison, 1959; Phillipson, 1959). However, there is evidence that successful reproduction can be achieved on much lower protein intakes (Klosterman, Buchanan, Bolin & Bolin, 1951; Klosterman, Bolin, Buchanan, Bolin & Dinusson, 1953). These workers obtained satisfactory results on daily intakes as low as 50 g digestible crude protein, but these findings have not been generally accepted. The diversity, therefore, between recommended standards

---

* Paper no. 1: *Br. J. Nutr.* (1966), **20**, 263.

and levels reported to give satisfactory results in feeding trials suggested that further investigation was necessary to establish more precisely the protein requirements of the ewe during pregnancy.

An investigation was therefore undertaken to study protein utilization in the ewe during late pregnancy with special emphasis on minimal requirements. Since the morphological growth data of Cloete (1939) and Wallace (1948) showed that the nutrient requirements for the products of conception were negligible up to mid-pregnancy, the detailed study was confined to the second half of pregnancy. The interacting effect of energy on nitrogen utilization as discussed by Chalmers (1961) was also recognized and two levels of energy intake were included in the study.

In an initial experiment carried out in 1963–4 two levels of digestible crude protein intake were given at two levels of energy. The results from this experiment showed that more useful information could be obtained by investigating a wider range of protein intakes, and a further experiment was carried out in 1964–5 using two lower protein intakes given at the same energy intakes as in the earlier experiment. The complete study included four levels of digestible crude protein intake ranging from approximately 27·5 to 110 g digestible crude protein per day for a 68 kg ewe. The two energy intakes adopted were maintenance plus 50% and maintenance plus 25%.

EXPERIMENTAL

Sixty-four Border Leicester × Scottish Blackface ewes aged between 3 and 4 years were used. The ewes were mated at pasture over a period of approximately 3½ months and the service dates recorded each day. Five weeks after service the ewes were selected in groups of four and brought indoors. Care was taken to select animals of similar weight and condition, and ewes of approximately 68 kg body-weight were used. The selection and management of the ewes were standardized between experiments. The ewes were given medium quality hay *ad lib.* for a period of 1 week to provide a gradual change from grass to concentrate feeding. After the preliminary feeding period the ewes within each group were allocated at random to one of four dietary treatments; these were treatments 1, 2, 5 and 6 in 1963–4 and treatments 3, 4, 7 and 8 in 1964–5. Details of the experimental treatments are given in Table 1.

Diets. The composition of the diets and the feeding scale are presented in Table 2. The feeding scale was based on the metabolic body-weight of each animal at the end of the preliminary feeding period and was kept constant throughout pregnancy. The diets aimed to provide as nearly as possible an equal daily fibre intake. The intakes of fibre ranged from 12·4 to 14·0 g/kg $W^{0.73}$, with a mean of 13·0 g/kg $W^{0.73}$ per day, where W = body-weight. The high and low energy intakes were achieved by adjusting the amount of concentrate given and by altering the proportions of high-energy and low-energy cereals in the diets.

Protein was given at four levels, P1–P4. In order to obtain the lower levels of N intake and maintain energy intake, it was necessary to use maize starch in the two lowest-protein diets at each energy level. Field-cured hay was a constituent of all the diets; the hay used in the diets containing maize starch was of slightly poorer quality than that used in the cereal diets and had a higher fibre content to balance the fibre deficit of the maize starch. The diets were intended to supply approximately 150 and 125 kcal metabolizable energy/kg $W^{0.73}$ daily on the high and low energy intakes respectively. These levels represent 150 and 125% respectively of the maintenance requirement of dry sheep estimated by Phillipson (1959) from the results obtained by Blaxter & Graham (1955).

Table 1. *Dietary treatments*

Treatment no.	Estimated energy intake	Estimated daily contribution of apparently digestible crude protein (g/68 kg ewe)	Symbol used to denote the level of protein intake
1	High energy (E1) 150 kcal ME/kg $W^{0.73}$	110·0	P1
2		82·5	P2
3		55·0	P3
4		27·5	P4
5	Low energy (E2) 125 kcal ME/kg $W^{0.73}$	110·0	P1
6		82·5	P2
7		55·0	P3
8		27·5	P4

ME, metabolizable energy; *W*, body-weight.

Table 2. *Composition of diets and feeding scales*

Constituent	Treatment no. 1	2	3	4	5	6	7	8
Chopped hay (%)	56·2	56·2	53·0	53·0	66·6	66·6	64·0	65·0
Flaked maize (%)	30·0	26·3	—	—	—	—	—	—
Flaked barley (%)	5·0	16·2	—	—	—	—	—	—
Rolled oats (%)	—	—	—	—	21·7	30·8	—	—
Maize starch (%)	—	—	40·0	44·0	—	—	29·0	33·0
Soya-bean meal (%)	8·8	1·3	7·0	3·0	11·7	2·6	7·0	2·0
Mineral supplement* (lb/100 lb conc. mix)	+2·5	+2·5	+2·5	+2·5	+2·5	+2·5	+2·5	+2·5
Vitamin supplement† (lb/100 lb conc. mix)	+0·25	+0·25	+0·25	+0·25	+0·25	+0·25	+0·25	+0·25
Crude protein content (%)	11·0	8·6	6·8	5·1	12·4	9·4	7·7	5·5
Metabolizable energy (kcal/g)	2·25	2·15	2·25	2·14	2·17	2·13	1·92	2·00
Feeding rate (g/kg $W^{0.73}$ daily)	73·4	73·4	69·3	68·8	61·9	61·9	57·3	56·4

* Declared composition: P 5·4%, Ca 25·0%, NaCl 25·0%, Fe 0·3%, Mn 1000 ppm, Co 100 ppm, I 200 ppm.

† Declared composition: 800000 i.u. vitamin A/lb and 200000 i.u. vitamin D_3/lb.

Management. The ewes were penned individually and given two equal feeds at 09.00 and 17.00 h daily. Hay and concentrates were given separately. The experimental diets were offered for a 4-week period before the beginning of the detailed investigation at 10 weeks gestation. This period allowed the rumen flora to become fully adjusted to the experimental treatments. At 16 weeks gestation all the ewes were given 200000 i.u. vitamin D_3 intramuscularly.

N balances and chemical analysis. N balances were carried out on five replicates of each treatment at three stages of gestation, 10–12, 14–16 and 18–20 weeks. The balance procedure and chemical analysis were the same as those described by Robinson & Forbes (1966). The intakes of metabolizable energy were calculated from the intakes of gross digestible energy and the protein contents of the diets, using the factors suggested by Blaxter, Clapperton & Martin (1966).

Statistical analysis. The confounding of protein treatments with experiments restricted the statistical comparisons which could be made. The protein levels within each experiment were compared independently and then the combined effect of protein levels was compared between experiments. Confounding did not, however, affect the main comparisons of the effects of energy intake and stage of gestation. The main protein and energy effects and protein × energy interactions were tested for significance using the variance between ewes within treatments. Where the variation due to interactions was less than the variation between ewes within treatments, the former source of variation was accumulated with the latter to provide a better estimate of the residual variance. The stage of gestation effects and their interactions were tested using the subplot error variance, i.e. the variation (between ewes within treatments) × stages. Again, if the variation of individual interactions was less than the subplot error variation it was accumulated with the latter to test the main effects.

Table 3. *Daily nutrient intakes at different times* ($S1$–$S3$) *during gestation*

(Mean values for five ewes per treatment)

	Dry-matter intake (g/kg $W^{0.73}$)			Crude protein intake (g/kg $W^{0.73}$)			Metabolizable energy intake† (kcal/kg $W^{0.73}$)		
Treatment*	10–12 weeks (S1)	14–16 weeks (S2)	18–20 weeks (S3)	10–12 weeks (S1)	14–16 weeks (S2)	18–20 weeks (S3)	10–12 weeks (S1)	14–16 weeks (S2)	18–20 weeks (S3)
1 E1 P1	57·6	52·3	48·6	7·43	7·21	6·70	153	148	127
2 E1 P2	55·1	53·2	50·1	5·60	5·54	5·41	145	136	129
3 E1 P3	56·3	51·7	46·3	4·48	4·25	3·81	132	131	120
4 E1 P4	54·4	53·6	46·9	3·29	3·31	2·84	137	133	119
5 E2 P1	50·3	47·9	46·8	7·35	7·18	7·06	132	125	120
6 E2 P2	50·0	48·3	43·8	5·52	5·40	5·22	134	120	110
7 E2 P2	47·3	45·5	44·0	4·28	3·96	3·99	105	107	99
8 E2 P4	46·5	44·4	39·5	2·98	2·84	2·48	105	112	91

* See Table 1. † Mean values for three ewes per treatment.

RESULTS

The mean daily nutrient intakes at the three stages of gestation are given in Table 3. The mean intake of dry matter with the different protein intakes within each energy feeding level varied slightly, particularly in late gestation. This variation was reflected in the intakes of metabolizable energy within the high- and low-energy treatments. There was a decrease in the nutrient intake/kg $W^{0.73}$ as pregnancy advanced. The daily mean crude protein intakes were 7·16, 5·45, 4·13 and 2·96 g/kg $W^{0.73}$ with the four diets of varying protein content, and the mean metabolizable energy intakes were 134 and 113 kcal/kg $W^{0·73}$ daily with the high and low energy intakes respectively.

The mean nutrient digestibilities and statistical comparisons are shown in Table 4. There were no protein × energy or stage × treatment interactions in dry-matter digestibility. The differences between protein levels within experiments were not significant but there was a highly significant difference between experiments. However, in all instances a higher digestibility was associated with the higher protein intake. The mean digestibility of the dry matter of the high-energy diet was significantly

Table 4. *Digestibilities of nutrients at different times* (S1–S3) *during gestation*

(Mean values for five ewes per treatment)

Treatment*	Dry-matter digestibility (%)† 10–12 weeks (S1)	Dry-matter digestibility (%)† 14–16 weeks (S2)	Dry-matter digestibility (%)† 18–20 weeks (S3)
1 E1 P1	73·6	75·2	75·5
2 E1 P2	73·6	73·0	72·4
3 E1P3	69·9	69·2	71·4
4 E1 P4	68·7	69·7	70·9
5 E2 P1	72·5	70·6	71·5
6 E2 P2	71·3	69·2	68·2
7 E2 P3	66·5	68·7	68·2
8 E2 P4	66·4	68·1	67·7
SE of treatment mean	±1·8	±1·2	±1·3

* See Table 1.

†	
P1, P2	NS
P3, P4	NS
(P1 + P2) > (P3 + P4)	$P < 0·001$
E1 > E2	$P < 0·001$
S1, S2, S3	NS

	Crude protein digestibility (%)‡ 10–12 weeks (S1)	Crude protein digestibility (%)‡ 14–16 weeks (S2)	Crude protein digestibility (%)‡ 18–20 weeks (S3)
1 E1 P1	62·1	64·8	65·2
2 E1 P2	54·0	54·6	56·8
3 E1 P3	43·5	45·7	47·4
4 E1 P4	27·8	32·5	33·7
5 E2 P1	70·7	71·1	71·7
6 E2 P2	61·6	62·0	61·6
7 E2 P3	48·0	48·5	46·4
8 E2 P4	32·0	31·2	31·4
SE of treatment mean	±1·9	±1·7	±1·5

‡	
P1 > P2	$P < 0·001$
P3 > P4	$P < 0·001$
(P1 + P2) > (P3 + P4)	$P < 0·001$
(P1 + P2) on E1 < (P1 + P2) on E2	$P < 0·01$
(P3 + P4) on E1, (P3 + P4) on E2	NS
E1 < E2 at S1 and S2	$P < 0·01$
E1, E2 at S3	NS
S1, S2, S3	NS

NS, not significant.

higher ($P < 0·001$) than that of the low-energy diet at all stages of gestation. There were significant (protein between experiment) × energy ($P < 0·001$), i.e. {(P1 + P2) *v.* (P3 + P4)} × energy, and energy × stage of gestation ($P < 0·05$) interactions in the apparent digestibility of crude protein. The protein and energy effects were therefore compared separately for each energy intake and stage of gestation. The digestibility of crude protein was significantly affected by protein intake in both experiments ($P < 0·01$). There was a significant difference in crude protein digestibility between the main energy effects at 10–12 weeks and at 14–16 weeks, but the difference at 18–20 weeks, although in the same direction, was not significant. Stage of gestation had no significant effect on the apparent digestibility of dry matter or crude protein.

Table 5. *Nitrogen balance results (g/kg* $W^{0·73}$ *daily) at different times (S1–S3) during gestation*

(Mean values for five ewes per treatment)

Stage of gestation	Treatment*	N intake	Faecal N	Apparently digested N	Urinary N	Retained† N	Percentage of apparently digested N retained
10–12 weeks (S1)	1 E1 P1	1·189	0·451	0·738	0·569	0·169	23·3
	2 E1 P2	0·896	0·413	0·483	0·338	0·145	28·7
	3 E1 P3	0·717	0·405	0·312	0·235	0·077	25·6
	4 E1 P4	0·526	0·378	0·148	0·137	0·011	6·2
	5 E2 P1	1·176	0·343	0·833	0·693	0·140	16·4
	6 E2 P2	0·883	0·337	0·546	0·444	0·102	18·1
	7 E2 P3	0·685	0·346	0·339	0·280	0·059	18·2
	8 E2 P4	0·476	0·324	0·152	0·168	−0·016	−10·9
SE of treatment mean		±0·041	±0·019	±0·031	±0·026	±0·024	±6·7
14–16 weeks (S2)	1 E1 P1	1·154	0·407	0·747	0·501	0·246	32·9
	2 E1 P2	0·886	0·404	0·482	0·283	0·199	41·4
	3 E1 P3	0·680	0·368	0·312	0·236	0·076	24·4
	4 E1 P4	0·529	0·366	0·163	0·134	0·029	16·0
	5 E2 P1	1·148	0·332	0·816	0·639	0·177	21·9
	6 E2 P2	0·864	0·329	0·535	0·386	0·149	27·9
	7 E2 P3	0·634	0·327	0·307	0·261	0·046	17·8
	8 E2 P4	0·454	0·314	0·140	0·146	−0·006	−4·6
SE of treatment mean		±0·020	±0·017	±0·017	±0·019	±0·015	±4·2
18–20 weeks (S3)	1 E1 P1	1·072	0·375	0·697	0·387	0·310	44·6
	2 E1 P2	0·866	0·374	0·492	0·207	0·285	58·1
	3 E1 P3	0·610	0·321	0·289	0·169	0·120	41·2
	4 E1 P4	0·454	0·302	0·152	0·118	0·034	23·0
	5 E2 P1	1·129	0·320	0·809	0·526	0·283	35·2
	6 E2 P2	0·835	0·313	0·522	0·318	0·204	38·7
	7 E2 P3	0·639	0·342	0·297	0·233	0·064	22·6
	8 E2 P4	0·397	0·271	0·126	0·121	0·005	3·2
SE of treatment mean		±0·027	±0·016	±0·024	±0·024	±0·018	±3·3

* See Table 1.

† P1, P2 NS E1 > E2 $P < 0·05$
P3 > P4 $P < 0·05$ S3 > S2 and S1 $P < 0·01$
(P1 + P2) > (P3 + P4) $P < 0·001$ S1 and S2 NS
NS, not significant.

The mean N balances are given in Table 5. The mean intakes of apparently digested N were lower on all the treatments than was originally intended. Faecal N was significantly lower ($P < 0.001$) with the low energy intake than with the high energy intake. There were no significant protein × energy or treatment × stage of gestation interactions. The results were therefore pooled to compare main effects. In both experiments N retention was lower on the lower protein intake. Although the mean retention on P1 was approximately 20% higher than on P2 the difference was not significant. The difference between P3 and P4 was significant ($P < 0.05$), and the difference between protein levels between experiments, i.e. (P1 + P2) *v.* (P3 + P4), was also highly significant ($P < 0.001$).

There was a significantly higher N retention on the high energy intakes at all stages of gestation ($P < 0.05$). The overall mean retentions during the second half of gestation were 0·142 and 0·100 g/kg $W^{0.73}$ per day for the high and low energy diets respectively. There was a significantly linear increase in N retention as pregnancy advanced. The overall mean N retentions were 0·086, 0·114 and 0·163 g/kg $W^{0.73}$ for 10–12, 14–16 and 18–20 weeks gestation respectively.

In order to assess changes in the efficiency of N utilization within the body the percentage of apparently digested N retained was computed for each ewe at each stage of gestation (Table 5) and the values were analysed statistically. There were no significant protein × energy or treatment × stage of gestation interactions. Protein intake, energy intake and stage of gestation had highly significant ($P < 0.001$) effects on the efficiency of N utilization. The mean efficiency of N utilization on P1 was lower than that obtained on P2 but the difference was not significant. The efficiency on P2 was higher than on P3, which in turn was significantly higher than on P4. This change in pattern of efficiency of N utilization between protein levels was further studied by considering the overall pattern of efficiency at the four protein levels. A highly significant quadratic relationship ($P < 0.001$) between efficiency and apparently digested N intake was obtained. It is accepted that this relationship includes random variation between experiments. Since experimental animals, techniques and management were standardized between experiments the effect of these factors was considered to be small compared with the contrasting response to the main treatments within each experiment. Regression relationships for each energy level and each stage of gestation are presented in Table 6. A quadratic model significantly reduced deviations from the linear form for each of the energy and stage of gestation groupings. Regression coefficients both for the individual regressions and the combined energy and stage of gestation regressions did not differ significantly amongst themselves, when an overall quadratic relationship was fitted to the results.

The overall regression equation $Y = -18.3 + 187.8X - 158.2X^2$, where Y = the retained N expressed as a percentage of the apparently digested N and X = the apparently digested N, is represented diagrammatically in Fig. 1. The point on the graph where retained N expressed as a percentage of apparently digested N is at a maximum represents the point of maximum net efficiency of N utilization. At this point the slope of the regression is zero. Since the slope can be calculated by differentiating the quadratic equation with respect to X, the intake of apparently digested N at which maximum efficiency of N utilization occurred can be obtained. In order to study changes in the level of apparently digested N at which maximum efficiency occurred,

Table 6. *Relationships in pregnant ewes between retained nitrogen as a percentage of apparently digested N (Y) and apparently digested N (X)*

Regression	Regression equation	Significance level	Multiple correlation coefficient	Residual standard error
High energy intake	$Y = -13{\cdot}9+205{\cdot}0X-185{\cdot}9X^2$	***	0·74	±1·33
Low energy intake	$Y = -24{\cdot}8+184{\cdot}9X-149{\cdot}0X^2$	***	0·75	±1·50
10–12 weeks gestation	$Y = -25{\cdot}8+170{\cdot}6X-137{\cdot}0X^2$	***	0·65	±2·23
14–16 weeks gestation	$Y = -14{\cdot}5+168{\cdot}8X-143{\cdot}8X^2$	***	0·71	+1·60
18–20 weeks gestation	$Y = -15{\cdot}4+232{\cdot}1X-204{\cdot}7X^2$	***	0·86	±1·36
Overall	$Y = -18{\cdot}3+187{\cdot}8X-158{\cdot}2X^2$	***	0·74	±1·08

In all the equations the quadratic term accounts for significantly more variation than the linear model.
*** $P < 0{\cdot}001$.

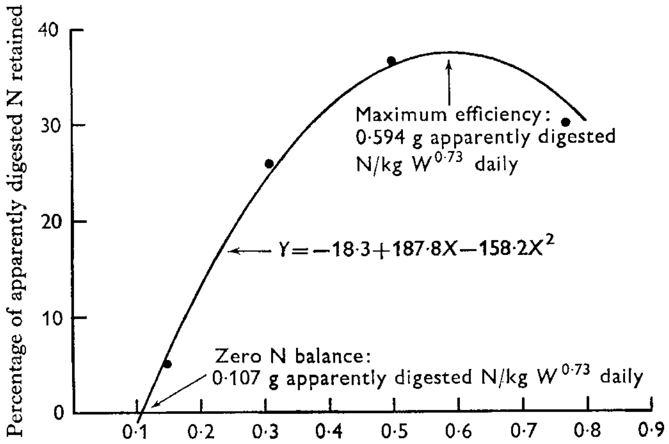

Fig. 1. Relationship in pregnant ewes between percentage of apparently digested nitrogen retained (*Y*) and apparently digested nitrogen (*X*). ●, protein treatment means.

values were calculated for the equations in Table 6. The results presented in Table 7 show that the intake of apparently digested N corresponding to maximum efficiency decreased with advancing pregnancy. There was a decrease from 0·623 g/kg $W^{0\cdot73}$ per day at mid-pregnancy to 0·567 g/kg $W^{0\cdot73}$ per day just before parturition. Energy intake also had an effect, the intake of apparently digested N at maximum efficiency for the high and low energy intakes being 0·551 and 0·620 g/kg $W^{0\cdot73}$ per day respectively.

The levels of N retained at maximum efficiency were calculated and are also given in Table 7. The level of N retained at maximum efficiency was 0·170 g/kg $W^{0\cdot73}$ per day at 10–12 weeks and increased to 0·286 g/kg $W^{0\cdot73}$ per day at 18–20 weeks. The levels from the pooled regressions for high and low energy intakes were 0·235 and 0·202 g/kg $W^{0\cdot73}$ per day respectively.

The requirement of apparently digested N for zero N balance was also calculated for each stage of gestation and each energy intake from the equation presented in Table 6. The values are also presented in Table 7. The requirement for N equilibrium decreased from 0·176 g/kg $W^{0\cdot73}$ at mid pregnancy to 0·071 g/kg $W^{0\cdot73}$ before parturition. The requirements for zero N balance for the overall high and low energy intakes were 0·072 and 0·153 g/kg $W^{0\cdot73}$ per day respectively.

Table 7. *Effect of energy intake and stage of gestation on nitrogen balance indices in pregnant ewes*

(Calculated from regressions presented in Table 6)

	Intake of apparently digested N required for maximum retention efficiency (g/kg $W^{0\cdot73}$ per day)	Level of N retained at maximum efficiency (g/kg $W^{0\cdot73}$ per day)	Intake of apparently digested N required for zero N balance (g/kg $W^{0\cdot73}$ per day)
High energy intake	0·551	0·235	0·072
Low energy intake	0·620	0·202	0·153
10–12 weeks gestation	0·623	0·170	0·176
14–16 weeks gestation	0·587	0·206	0·093
18–20 weeks gestation	0·567	0·286	0·071
Overall	0·594	0·223	0·107

DISCUSSION

The diets used in this investigation were intended to supply a range of crude protein intakes at two levels of energy intake, while reducing to a minimum extraneous factors which might affect N utilization. The association between fibre intake and acetic acid production in the rumen is well known (Annison & Lewis, 1959) and, in view of the findings of Armstrong & Blaxter (1957) on the effect of acetic acid on N utilization, fibre intakes were standardized as far as possible between diets. Since this investigation began, Elliott & Topps (1964) have shown that the dietary ratio of roughage to concentrate has a significant effect on N utilization. Although the effect is probably related to fibre intake, the roughage to concentrate ratios within energy intakes in the present study were reasonably constant and the difference between energy intakes was small. The mean contents of roughage in the high-energy and low-energy diets were 54·6 and 65·6% respectively, and judging from the results of Elliott & Topps (1964) the difference was too small to have any marked effect on N utilization. It was necessary to use a proportion of maize starch in the low-protein diets to ensure adequate energy intake. It was recognized that maize starch might affect N digestibility (Head, 1953*a*; Tagari, Dror, Ascarelli & Bondi, 1964) and for this reason the maize starch in the low-protein diets replaced the starchy cereal components of the higher-protein diets. This ensured that any interacting effect of starch on N utilization was minimal.

As pregnancy advanced there was a decrease in the intake of metabolizable energy per unit metabolic body-weight. This was due mainly to the fact that intake was based on the live weights of the ewes at 6 weeks of gestation and was not adjusted for increase in body-weight as pregnancy advanced. The total intake was kept constant because the work of Graham & Williams (1962) and Elliott & Topps (1963) had indicated that it would be difficult to maintain a constant intake per unit metabolic body-weight. Even at the level adopted, intake declined as pregnancy advanced and this was more pronounced on the low-protein diets. The weight of food left uneaten, expressed as a percentage of total food offered, increased from under 1% on all diets at 10–12 weeks gestation to 6·4% on the highest-protein diet and to 13·0% on the lowest-protein diet just before parturition. The intakes of metabolizable energy with the low-protein diets were lower than planned but, when compared with the values for basal metabolism given by Blaxter (1962), they were considered adequate.

The significant decrease in dry-matter digestibility between experiments may be attributed to a random difference between experiments. However it is of interest that, although differences between protein intakes within experiments were not significant, the mean values within each experiment were lower with the low protein intake. Burroughs, Gall, Gerlaugh & Bethke (1950) and Williams, Nottle, Moir & Underwood (1953) found a significant reduction in dry-matter digestibility with decreasing protein intake. These workers showed that the decrease was accompanied by a decrease in the numbers of rumen bacteria with low-protein diets. In the present study there was no significant change in dry-matter digestibility as gestation advanced, and this would suggest that the rumen bacteria had become adjusted to the diets before mid-pregnancy. This was as expected in view of the results of Lloyd, Peckham & Crampton (1956) and Stielau (1960) on the length of the preliminary feeding period required for dry-matter digestibility to reach a constant level after extreme changes in diet. The significantly higher dry-matter digestibility on the high-energy diets was due to the higher proportion of readily fermentable carbohydrate in these diets (Blaxter & Wainman, 1964).

The apparent digestibility of crude protein was significantly depressed on the high-energy diet. There was a difference in dry-matter intakes between the high- and low-energy diets, and the apparent digestibilities of crude protein were therefore corrected for metabolic faecal N using the regression method of Blaxter & Mitchell (1948). The levels of metabolic faecal N obtained (59 degrees of freedom) were 0·61 ± 0·01 and 0·70 ± 0·02 g/100 g dry matter consumed for the high- and low-energy diets respectively and these were significantly different ($P < 0·001$). The true digestibility of crude protein was therefore significantly lower on the high energy intake. It is not clear whether this was an overall effect of energy or the specific effect of maize starch as found by Head (1953*a*).

The apparent digestibility of crude protein declined more rapidly on the low-energy diets; the fall was 39·6 percentage units compared with 32·7 percentage units on the high-energy diets. This probably caused the significant protein × energy interaction in the apparent digestibility of crude protein and can be associated with the difference in the calculated metabolic faecal N between the two energy intakes. The difference would result in a disproportionate reduction in the apparent digestibilities which would be accentuated at the low levels of protein intake. The apparent digestibilities of dry matter and crude protein were not affected by stage of pregnancy. This result is similar to that found by Head (1953*b*) and supports the view of Thomson & Aitken (1959) that the apparently increased efficiency of the pregnant animal is not likely to be the result of increased efficiency in digestibility.

The abolute retention of N is the most important criterion of the adequacy of dietary N intake. Retention was not affected at any stage of gestation by the number of foetuses carried and this suggests that retention may not be entirely governed by demand. Rombauts (1959) has reported similar findings. The mean levels of N retained on the high- and low-energy diets were 0·142 and 0·100 g/kg $W^{0.73}$ respectively. The mean increase in retained N per kcal increase in metabolizable energy intake was 2 mg and varied from 1·3 mg at 10–12 weeks gestation to 2·5 mg just before parturition. This emphasizes the importance of energy intake on N utilization (Munro, 1964) and suggests that energy intakes higher than the generally accepted requirement of maintenance plus 25 % for late pregnancy may have a beneficial effect on N retention.

The levels of N retained at mid-pregnancy on intakes of 0·50 and 0·15 g apparently digested N/kg $W^{0.73}$ per day were similar to those obtained for non-pregnant ewes on comparable intakes (Robinson & Forbes, 1966). This would indicate that up to mid-pregnancy the demand for N is similar to that of the non-pregnant animal. The amount retained increased from 0·086 g N/kg $W^{0.73}$ at mid-pregnancy to 0·114 g N at 14–16 weeks and 0·163 g N at 18–20 weeks of gestation. The results of Klosterman *et al.* (1953) showed a similar pattern of increasing retention during gestation but the pattern occurred only on high protein intakes. In the present study the increase in N retention was accompanied by a corresponding decrease in urinary N output. It is apparent, therefore, that increased demand is met by increased efficiency in utilizing absorbed N rather than by increased absorption. Graham (1964) has also reported a decrease in urinary N output as pregnancy advances.

The mean percentages of apparently digested N retained were 29·1, 35·5, 25·2 and 5·6 on P1, P2, P3 and P4 respectively, and suggested that there was considerable variation in the efficiency of N utilization between dietary N intakes. In order to investigate these differences the regression relationship between the percentage of apparently digested N retained and the apparently digested N intake was used. The use of a regression in which the variable on one axis is the denominator of a ratio on the other axis may be questioned, but Sutherland (1965) used this relationship and concluded that it was a valid regression. It is accepted that the calculated response curve shown in Fig. 1 may not give precise estimates of the absolute pattern of N utilization owing to possible differences between experiments. However, relative comparisons of the pattern of N utilization on each energy intake and at each stage of gestation should not be invalidated.

The calculated mean intake of apparently digested N corresponding to maximum efficiency for the overall period from 10 weeks gestation to parturition was 0·594 g/kg $W^{0.73}$ per day. Although the mean retention of 0·223 g/kg $W^{0.73}$ per day for this period would appear adequate for foetal growth (Agricultural Research Council, 1965), the mean intake of apparently digested N was approximately 15 % less than the mean requirement recommended by Morrison (1959). It is, however, similar to the mean levels advocated by the National Research Council (1957) for the second half of pregnancy.

It was suggested earlier that the efficiency with which the pregnant animal utilizes digested N improves as pregnancy advances. The efficiency values calculated from the individual regressions for the three stages of pregnancy confirmed this. There was a decrease in the intake of apparently digested N required for maximum retention efficiency from 0·623 g/kg $W^{0.73}$ per day at 10–12 weeks to 0·567 g/kg $W^{0.73}$ per day at 18–20 weeks. The fact that the level of N retention at maximum efficiency increased from 0·170 to 0·286 g/kg $W^{0.73}$ per day during the same period further accentuates the increase in efficiency.

The increased efficiency was also reflected in a decrease from 0·176 g/kg $W^{0.73}$ per day at mid-pregnancy to 0·071 g/kg $W^{0.73}$ per day at parturition in the calculated intakes of apparently digested N for zero N balance. The requirement for zero N balance at mid-pregnancy was very similar to that obtained in non-pregnant ewes (Robinson & Forbes, 1966) and supports the findings of Graham (1964) that there is little change in N metabolism until mid-pregnancy. This finding may also indicate that the possible between-year variation had little effect on the regression relationship. The pronounced decrease in the equilibrium requirement in advanced pregnancy suggests that there may be some transfer of N from maintenance to productive purposes as pregnancy advances. The decrease in the requirement for zero N balance in advanced pregnancy may also indicate urea recycling (Houpt, 1959; Somers, 1961; Packett & Groves, 1965) in late pregnancy. The results also confirm the view of Moustgaard (1962) that in conditions of undernutrition the foetus has priority for amino acids.

The level of N intake required for maximum efficiency just before parturition was equivalent to 77 g apparently digestible crude protein per day for a 68 kg ewe. This is approximately 35% lower than generally accepted standards for late pregnancy (Thomson & Aitken, 1959; Morrison, 1959; Phillipson, 1959). The corresponding level of N retained was 0·286 g/kg $W^{0.73}$ per day (6·2 g for a 68 kg ewe) and was considerably higher than that reported by Klosterman *et al.* (1953) for a similar N intake. It does, however, correspond closely to the N requirements for foetal growth suggested by the Agricultural Research Council (1965).

The effect of energy intake on N retention is well known, and calculated N intakes and retentions at maximum efficiency for the two energy levels used in the present study are of interest. The overall mean intake of apparently digested N for maximum efficiency on the high energy intake was 0·551 g/kg $W^{0.73}$ per day compared with 0·620 on the low energy intake. The lower mean intake of apparently digested N on the high-energy diet was accompanied by an approximate 16% increase in the level of N retained and further emphasizes the importance of adequate energy intake in achieving maximum efficiency of N utilization in the pregnant ewe.

The authors thank Professor J. Morrison and the Trustees of The Agricultural Research Institute of Northern Ireland for the facilities provided for this work. They thank Mr W. H. Foster of the Department of Crop and Animal Husbandry for advice on statistical analyses and Mr R. Watt for technical assistance.

REFERENCES

Agricultural Research Council (1965). *The Nutrient Requirements of Farm Livestock.* No. 2, *Ruminants.* London: Agricultural Research Council.

Annison, E. F. & Lewis, D. (1959). *Metabolism in the Rumen.* London: Methuen and Co. Ltd.

Armstrong, D. G. & Blaxter, K. L. (1957). *Br. J. Nutr.* **11**, 247.

Blaxter, K. L. (1962). *Br. J. Nutr.* **16**, 615.

Blaxter, K. L., Clapperton, J. L. & Martin, A. K. (1966). *Br. J. Nutr.* **20**, 449.

Blaxter, K. L. & Graham, N. McC. (1955). *J. agric. Sci., Camb.* **46**, 292.

Blaxter, K. L. & Mitchell, H. H. (1948). *J. Anim. Sci.* **7**, 351.

Blaxter, K. L. & Wainman, F. W. (1964). *J. agric. Sci., Camb.* **63**, 113.

Burroughs, W., Gall, L. S., Gerlaugh, P. & Bethke, R. H. (1950). *J. Anim. Sci.* **9**, 214.

Chalmers, M. I. (1961). In *Digestive Physiology and Nutrition of the Ruminant,* p. 205 [D. Lewis, editor.] London: Butterworths.

Cloete, J. H. L. (1939). *Onderstepoort J. vet. Sci. Anim. Ind.* **13**, 417.

Elliott, R. C. & Topps, J. H. (1963). *J. Anim. Prod.* **5**, 269.

Elliott, R. C. & Topps, J. H. (1964). *Br. J. Nutr.* **18**, 245.

Graham, N. McC. (1964). *Aust. J. agric. Res.* **15**, 127.

Graham, N. McC. & Williams, A. J. (1962). *Aust. J. agric. Res.* **13**, 894.

Head, M. J. (1953*a*). *J. agric. Sci., Camb.* **43**, 281.

Head, M. J. (1953*b*). *J. agric. Sci., Camb.* **43**, 214.

Houpt, T. R. (1959). *Am. J. Physiol.* **197**, 115.

Klosterman, E. W., Bolin, D. W., Buchanan, M. L., Bolin, F. M. & Dinusson, W. E. (1953). *J. Anim. Sci.* **12**, 188.

Klosterman, E. W., Buchanan, M. L., Bolin, D. W. & Bolin, F. M. (1951). *J. Anim. Sci.* **10**, 257.

Lloyd, L. E., Peckham, H. E. & Crampton, E. W. (1956). *J. Anim. Sci.* **15**, 846.

Morrison, F. B. (1959). *Feeds and Feeding,* 22nd ed. Iowa: The Morrison Publishing Co.

Moustgaard, J. (1962). In *Nutrition of Pigs and Poultry,* p. 189. [J. T. Morgan and D. Lewis, editors.] London: Butterworths.

Munro, H. N. (1964). In *Mammalian Protein Metabolism.* Vol. 1, p. 381. [H. N. Munro and J. B. Allison, editors.] New York and London: Academic Press Inc.

National Research Council (1957). *Publs natn. Res. Coun., Wash.* no. 504.

Packett, L. V. & Groves, T. D. D. (1965). *J. Anim. Sci.* **24**, 341.

Phillipson, A. T. (1959). *Scientific Principles of Feeding Farm Livestock,* p. 105. London: Farmer and Stock-Breeder Publications Ltd.

Robinson, J. J. & Forbes, T. J. (1966). *Br. J. Nutr.* **20**, 263.

Rombauts, P. (1959). *C.r. hebd. Séanc. Acad. Sci., Paris,* **248**, 1859.

Somers, M. (1961). *Aust. J. exp. Biol. med. Sci.* **39**, 145.

Stielau, W. J. (1960). *S. Afr. J. agric. Sci.* **3**, 433.

Sutherland, T. M. (1965). *Biometrics* **21**, 739.

Tagari, H., Dror, Y., Ascarelli, I. & Bondi, A. (1964). *Br. J. Nutr.* **18**, 333.

Thomson, W. & Aitken, F. C. (1959). *Tech. Commun. Commonw. Bur. Anim. Nutr.* **20**.

Wallace, L. R. (1948). *J. agric. Sci., Camb.* **38**, 243.

Williams, V. J., Nottle, M. C., Moir, R. J. & Underwood, E. J. (1953). *Aust. J. biol. Sci.* **6**, 142.

Printed in Great Britain