STELLINGEN

1. In tegenstelling tot wat nu gebruikelijk is mogen rasverschillen bij eiwitwaardering voor schapen niet buiten beschouwing gelaten worden.

   Dit proefschrift

2. De gangbare opvatting dat de effectieve afbraak van nutriënten in de pens afneemt door koude stress is niet correct.

   Dit proefschrift

3. Onze maatschappij heeft patriarchale kenmerken, toch is de vrouw de belangrijkste doorgeefster van ons sociaal culturele erfgoed.

4. De economieën in het vergrijzende Westen van Europa kunnen op termijn alleen draaiende worden gehouden door (gast)arbeiders uit de toekomstige EU landen.

5. Het verbod op het gebruik van een levende aasvis is dieronvriendelijk.

6. De slogan "Sporten is gezond" heeft een beperkt waarheidsgehalte.

L.B.J. Šebek
Protein metabolism of prolific ewes during late gestation and early lactation

Wageningen, 17 oktober 2001

PROTEIN METABOLISM OF PROLIFIC EWES DURING LATE GESTATION AND EARLY LACTATION

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PROTEIN METABOLISM OF PROLIFIC EWES DURING LATE GESTATION AND EARLY LACTATION

L. B. J. Šebek

Proefschrift
ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. Dr. Ir. L. Speelman,
in het openbaar te verdedigen
op woensdag 17 oktober 2001
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Šebek, L. B. J., 2001. Protein metabolism of prolific ewes during late gestation and early lactation.
Introduction of prolific crossbred ewes and a new protein evaluation system for ruminants (the DVE/OEB system) necessitate a reconsideration of ewe feeding strategies. The objective of this thesis is to investigate the amount of protein digested in the small intestine (DVE), the efficiency by which the digested protein is used during late pregnancy and early lactation and to evaluate current Dutch protein feeding strategy. This feeding strategy was developed for ewes bearing one or two lambs, but it shows that prolific ewes yield approximately10% more DVE from the same feed. This is due to rumen degradation enhancement resulting in larger amounts of effectively degraded protein and synthesised microbial protein in prolific ewes. Winter shearing results in further increases in DVE yield. Current DVE maintenance requirements and production efficiencies are also valid for prolific ewes. The DVE requirements for prolific ewes, calculated from the measured protein production and efficiency of protein utilisation, are in agreement with the requirements derived from the feeding trials during late pregnancy and early lactation. Adaptation of the current protein feeding strategy is proposed for prolific crossbred ewes.

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Aan Karen,
Lotte , Maartje en Fleur

Voorwoord

Het experimentele onderzoek waarop dit proefschrift is gebaseerd werd van 1992 tot en met 1996 uitgevoerd bij ID TNO Diervoeding te Lelystad. De medewerkers die op welke wijze dan ook hebben meegewerkt aan dit onderzoek wil ik daarvoor hartelijk danken.
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Chapter 1

General Introduction

Introduction

Professional sheep husbandry in The Netherlands is characterised by lamb meat production. The financial outcome of sheep farming depends therefore on the annual lamb meat production per ewe and the price of lamb meat. Within normal market fluctuations, the price of lamb meat is related to the EUROP carcass classification. The commonly used Texel sheep breed is known to produce lamb carcasses with a high EUROP classification, which only leaves increase of the annual meat production per ewe to influence the financial outcome of sheep farming. This can be achieved by using prolific sheep breeds with potential for large litter sizes. However, lambs of prolific breeds are often characterised by relatively low EUROP carcass classifications, resulting in a lower economic value. The combination of large litter sizes and fast growing, heavy lambs with a high EUROP carcass classification has been achieved with crossbred ewes. These ewes are the result of crosses between a meat producing breed and a prolific breed (e.g. the Flevolander crossbred as Ile the France ♂ x Finnish Landrace ♀). Mating of these crossbred ewes to Texel rams minimises the loss in carcass quality of the lambs produced and the increases in litter size provide ample financial compensation. However, current feeding strategies for (Texel) ewes may be inadequate for crossbred ewes. When fed according to the current feeding strategies prolific ewes can have problems with their energy and protein supply during late gestation (Everts, 1990). Since energy requirements have been investigated by Everts (1990), the present research focuses on the protein requirements of the crossbred ewe.

Another reason to investigate protein feeding strategies for sheep is the introduction of a new Dutch protein evaluation system for ruminants, the DVE/OEB system (Tamminga et al., 1994). This protein evaluation system is a product of the growing concern of our society about the effects of intensive animal husbandry systems on the environment. The nitrogen (N) losses to the environment were high (e.g. 80% in dairy cows; Tamminga, 1992) due to a low gross efficiency of N utilisation. To be able to increase this efficiency without jeopardising production, a new protein evaluation system was developed as a tool to balance protein feeding with protein requirements by describing protein digestion and metabolism in more detail. However, the DVE/OEB system is designed primarily for protein evaluation for dairy cows and protein reference values of feedstuffs are therefore based on protocols using dairy cows. These reference values are also used for sheep on the assumption that rumen kinetics are comparable between sheep and cows (Everts, 1992). This assumption may be invalid since sheep and cows are usually fed with different diets and the diet fed is one of the most important sources of differences in rumen degradation kinetics (Huntington and Givens, 1995).

Protein requirements of sheep

The current protein requirements are based on ewes with relative small litter sizes and it is uncertain whether or not these requirements apply to crossbred ewes with large litter sizes. Protein requirements depend on the amount of protein needed for maintenance and production (i.c. protein accretion in foetuses and milk protein production), in combination with the efficiency by which feed protein is utilised for accretion in foetuses or for milk protein production. The feeding strategy for multiparous ewes aims to supply sufficient protein for production and maintenance of body weight over a production cycle. The periods with a high production level are late pregnancy and early lactation, both periods being important for lamb performance and thus the economics of sheep farming.
During late pregnancy the nutrient requirement increases rapidly with the increasing growth of the foetuses, whereas rumen volume is diminished by the growing uterus. As a result large litter sizes (>2) often coincide with a reduction in feed intake during late gestation and with low lamb birth weights (Orr and Treacher, 1984). Low lamb birth weight is related to an increased lamb mortality before weaning (Hinch et al., 1985) and impinges on postnatal growth and productivity (Bell, 1992). Lamb birth weight can be positively influenced by nutrition during pregnancy. A positive linear relationship between both energy- and protein intake and lamb birth weight exists (Robinson and Forbes, 1967). Extra protein and energy intake during late pregnancy may result in a substantial increase in lamb birth weight (Stephenson and Bird, 1992). An alternative way to increase birth weight is to shear ewes 6 to 8 weeks prior to parturition. In practice this shearing occurs during winter and thus the pregnant ewes have to be housed indoors to protect them from harsh weather conditions. Another advantage of winter shearing is that it compensates for the decrease in feed intake due to a large litter size by increasing the rumen turnover rate.
The protein (and energy) intake of lambs during early lactation is essential for lamb growth performance to slaughter. The amount of protein needed for milk production depends on the amount of protein excreted with milk and the efficiency by which feed protein is used for milk protein production. The average efficiency of milk protein production in ewes is 0.68 (ARFC, 1995), but varies between 0.62 and 0.94 (Everts, 1992). An increased protein ingestion may also result in an increased milk production. This relationship between protein ingestion and milk (protein) production is linear up to an optimal plateau (Gonzales et al., 1984). This plateau level increases with increasing energy ingestion levels (Robinson, 1980).

The DVE/OEB system used for sheep

According to the DVE/OEB system the DVE value of a feedstuff is based on the amount of rumen undegraded feed protein and the amount of microbial protein that is digested in the small intestine (SI). The amount of undegraded feed protein entering the SI is calculated as the complement of the amount of protein that is effectively degraded in the rumen. The

effective rumen degradability (ERD) of feed protein is calculated from a combination of rumen degradation characteristics measured with the nylon bag technique (soluble and undegradable fraction and the degradation rate Kd of the potentially degradable fraction) and an assumed appropriate rumen passage rate (for concentrates and roughages 6 and 4.5 % per hour respectively). The amount of microbial protein entering the SI is calculated from the amount of organic matter (OM) that is fermented in the rumen (FOM) in combination with an assumed appropriate efficiency for microbial protein synthesis (150 g microbial protein per kg FOM). FOM is calculated as the apparently digestible OM (DOM) minus the components from which it is assumed that no energy becomes available for rumen microbes.

Rumen degradation characteristics are susceptible to a number of influences. Apart from the protocol used, the diet fed is one of the most important sources of variation in results of the nylon bag technique (Huntington and Givens, 1995). It is therefore likely that cows and sheep differ in rumen degradation characteristics. In sheep not only diet, but also breed (De Waal, 1995) and midwinter shearing (Kennedy et al., 1986) can influence rumen degradation kinetics. These differences may be due to differences in rumen pH, (critical) particle size, amount of microbes per unit of volume and microbial vitality. The impact of differences in rumen degradation characteristics on protein evaluation is not clear unless rumen passage rate (Kp) is taken into account. Most protein evaluation systems (including the DVE/OEB system) assume constant Kp's for protein in roughages and concentrates. This assumption is suitable for common protein evaluation, but becomes questionable when the animals are exposed to cold (Kennedy et al., 1986). Besides, it may be questionable to use the same Kp for sheep and dairy cows, because of differences in the amount of nutrients recycling in the rumen (Nocek, 1988).

Outline of this thesis

The aim of this thesis was to investigate both protein requirements in crossbred ewes and DVE evaluation for sheep in order to be able to validate current DVE recommendations for crossbred ewes. Therefore, knowledge was required concerning rumen degradation kinetics in dairy cows and sheep, in Texel and crossbred ewes and in unshorn and shorn crossbred ewes (Chapter 2). The effect of winter shearing on the effective rumen degradability of protein and organic matter as well as on DVE evaluation for crossbred ewes had to be investigated (Chapter 3). Furthermore, the use of the tables of reference DVE values of feedstuffs for sheep had to be validated (Chapter 3). Knowledge was also required concerning the (efficiency of) utilisation of the absorbed protein and the amount of protein needed for maintenance and production during late gestation (Chapter 4) and early lactation (Chapter 6). For ewes during late pregnancy this knowledge should be available for winter shorn ewes as well as for unshorn ewes (Chapter 5). Finally, the protein requirements had to be validated in feeding trials with crossbred ewes during late gestation (Chapter 5) and early lactation (Chapter 6).

Aspects of DVE evaluation for sheep are discussed and combined with the results of production and utilisation of protein for prolific crossbred ewes (Chapter 7). The result is a proposal to adapt DVE requirements for crossbred ewes during late gestation and early lactation (Chapter 7).

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Chapter 2

In situ rumen degradation of dry matter and crude protein in ewes and dairy cows

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Abstract

Comparative studies were performed on the in-situ rumen degradation rate (Kd) and the in-situ rumen undegradable residue (U) of dry matter (DM) and crude protein (CP) in sheep and dairy cows. The effect of different treatments for sheep were investigated, together with the validity of using cow based reference protein values of feedstuffs for sheep. The effect of different breeds (Texel ewes and crossbred ewes), dietary roughage:concentrate ratio and shearing (mild cold exposure) were investigated for sheep.

The in-situ rumen incubations were performed in accordance with standard operational procedures that were comparable for both species. The feeds under consideration were hay and concentrates.

Cows displayed lower (DM 53%, CP 86%) Kd's than sheep for concentrates. For hay a clear trend between cows and sheep was not observed, but cows displayed 40% lower Kd's than Texel ewes and 30% higher Kd's than crossbred ewes. For concentrates U levels did not differ between species, but for hay cows had 45% higher U's than Texel ewes. Texel ewes showed 45% higher Kd and 35% lower U for DM and CP than crossbred ewes. Dietary roughage:concentrate ratio in sheep diets did not influence the Kd's and U's of the feeds under consideration. Shearing of crossbred ewes increased Kd of CP by 25% and reduced U for DM and CP by 20% and 24%.

In conclusion, different in-situ rumen degradation rates were found between dairy cows and sheep. This probably makes using reference protein values of feedstuffs invalid for sheep, since they are based on data for cows. Differences (Kd and U) were also found between sheep breeds and between unshorn and shorn crossbred ewes (mild cold exposure). No effect on rumen degradation kinetics could be attributed to dietary roughage:concentrate ratio.

Keywords : dry matter, protein, rumen degradation, ewes, dairy cows

Introduction

Protein evaluation systems (Madsen, 1985 ; Vérité, Michalet-Doreau, Chapoutot, Peyraud and Poncet, 1987 ; Tamminga, van Straalen, Subnel, Meijer, Steg, Wever and Blok, 1994) were designed for dairy cows and were intended to allow estimation of the protein value of feedstuffs for these animals. Tables of reference protein values of feedstuffs are therefore based on protocols using dairy cows and these are used for sheep (Everts, 1992) on the assumption that rumen kinetics are comparable between sheep and cows. This assumption was made despite sheep and cows usually being fed different diets ; the diet of the animal being one of the most important sources of variation in results of *in situ* techniques (Lindberg, 1985 ; Nocek, 1988 ; Huntington and Givens, 1995). The validity of using cow-based reference protein values of feedstuffs for sheep was tested by comparing *in situ* kinetics of feeds incubated in sheep and cows fed their species specific diets.

In sheep not only diet (during pregnancy the roughage:concentrate ratio in ewe diets increases), but breed (De Waal, 1995) and whether they have been shorn (temperature effect) (Kennedy,

Christopherson and Milligan, 1976 ; Kennedy, Young and Christopherson, 1977 ; Weston, 1983 ; Westra and Christopherson, 1976) can also influence rumen degradation kinetics. At present no comparative data with respect to rumen degradation kinetics between meat sheep breeds and prolific sheep breeds and with respect to any influence of cold exposure on rumen degradation rates is available.

The present study investigates the validity of current reference protein values of feedstuffs for sheep. A number of experiments on *in situ* rumen degradation kinetics were performed, including comparisons of rumen degradation kinetics of feeds as measured in ewes with reference values (measured in dairy cows according to standard procedures), of ewes fed diets with different roughage:concentrate ratio, of ewes of different breeds and of shorn and unshorn ewes.

Materials and methods

Animals

Reference values for the degradation rates (Kd) and the undegradable fractions (U) of dry matter (DM) and crude protein (CP) were estimated using dairy cows according to standard ID-DLO procedures (Hindle, Steg, Van Vuuren and Vroons-de Bruin, 1995). Dry Holstein Friesian cows (live weight 673 ± 44 kg) were fitted with a rumen cannula of 10 cm internal diameter (Bar Diamond Inc., Idaho, USA) and housed at the ID-DLO experimental farm.
Barren ewes, either of the meat producing Texel breed (live weight 72 ± 6.3 kg) or of a prolific crossbred (Ile de France ♂ x Finnish Landrace ♀ ; live weight 71 ± 9.0 kg) were used. The ewes were fitted with a rumen cannula of 6.5 cm internal diameter (Bar Diamond Inc., Idaho, USA) and were kept indoors at the ID-DLO experimental farm.

Feedstuffs

The *in situ* rumen incubation experiments were performed with *Lolium perenne* hay (H) and two concentrates, one with a high protein content (CH) and one with a low protein content (CL). Table 2.1 provides information concerning the soluble fraction of DM and CP and the chemical composition of the feeds used for *in situ* rumen incubation. The hays and concentrates used each year differed but were of comparable feeding value. In the last experimental year (1996) the *in situ* rumen incubations involved hay and a commercially available concentrate for sheep (CC). Table 2.2 gives the composition of the concentrates used.
The concentrate samples were ground to pass through a 3 mm sieve and the hay incubation samples were treated with liquid nitrogen and then subsequently chopped in a cutter to an average particle length of approximately 1 cm. After mixing sub samples were taken to fill the coded nylon bags.

Table 2.1
Characteristics and chemical composition (g/kg DM and DM in g/kg product) of the feeds[†] used for in situ rumen incubation

	H				CL			CH			CC
Component	1	2	3	4	1	2	3	1	2	3	
S-DM[‡]	0.16	0.15	0.22	0.19	0.39	0.42	0.45	0.31	0.38	0.36	0.45
S-CP[‡]	0.27	0.25	0.27	0.22	0.26	0.32	0.40	0.17	0.18	0.18	0.47
dOM_TT[§]	0.71	0.71	0.74	0.74	0.88	0.83	0.84	0.85	0.84	0.85	0.81
Dry matter	860	894	865	860	888	862	863	881	881	876	902
Ash	101	107	100	100	56	67	68	66	77	73	107
Crude protein	182	192	141	148	70	102	118	366	355	352	187
Crude fat	28	21	23	24	30	58	65	48	58	60	40
Crude fibre	285	296	274	257	162	82	88	67	58	62	145
Sugar	---	---	---	---	70	66	90	69	91	103	90
Starch	---	---	---	---	312	344	345	136	143	147	103
Neutral detergent fibre	562	602	526	562	259	166	156	168	124	125	316

[†]H=hay, CL=concentrate low protein, CH=concentrate high protein and CC=commercially available concentrate
[‡]soluble fraction of DM (S-DM) and CP (S-CP)
[§] digestibility of organic matter (Tilley and Terry, 1963)

Table 2.2
Concentrate composition (g/kg)

	CL[†]			CH[‡]			CC[§]
	1	2	3	1	2	3	1
Feedstuff							
Animal fat	11	38	40	20	29	30	--
Barley	200	210	220	200	200	200	--
Casein	3	--	--	122	--	--	--
Cellulose	146	30	30	--	8	12	--
Citrus pulp	100	100	100	60	50	41	--
Coconut expeller	--	--	--	--	--	--	93
Linseed	20	20	20	20	20	20	--
Linseed solvent extracted	--	--	--	--	--	--	10
Lucerne meal	--	--	--	--	--	--	147
Lupins	--	--	--	--	--	--	75
Maize gluten feed	--	--	--	--	--	--	125
Maize gluten meal	--	--	--	150	100	117	--
Minerals / vitamins	30	29	37	28	26	27	40
Molasses, cane	40	50	50	20	50	40	60
Palm kernel expeller	--	--	--	--	--	--	174
Potato pulp	--	--	--	--	--	--	100
Potato starch	250	283	253	--	--	---	--
Soya bean solvent extracted	--	--	--	200	350	350	17
Soya bean hulls	--	--	--	--	--	--	100
Sugar beet pulp	200	200	200	180	102	100	59
Wheat gluten meal	--	40	50	--	65	63	--

[†]concentrate low protein
[‡]concentrate high protein
[§]commercially available concentrate

Incubation procedure and chemical analyses of incubation residues

The incubation procedure was similar to the one described by Hindle *et al.* (1995). Each nylon bag (polyamide, 190x100 mm, pore size 41 Fm, porosity 30%) which was to be incubated for less than 72 hours was filled with 5 g DM (15.4 mg cm^{-2}) and those intended for longer incubations with 7.5 g DM (23.1 mg cm^{-2}). This was to ensure sufficient residual material for chemical analysis. Per incubation time two bags per feed were incubated *in situ*. The bags were placed in the rumen one hour after morning feeding and were incubated for 0, 2, 4, 8, 12, 24, 48, 72 and 336 hours. The rumen suspension device was a solid PVC cylinder with a diameter

of 6.0 and 8.0 cm, a height of 9.0 and 12.0 cm and a weight of approximately 350 and 800 grammes for sheep and cows respectively. During *in situ* incubation the cylinder was attached to the rumen cannula by a nylon cord with a length of approximately 25 cm for sheep and 75 cm for cows. Each bag was attached to the cylinder by a nylon cord of approximately 12.5 cm for sheep and 25 cm for cows. The maximum number of nylon bags present in the rumen was restricted to 6 in sheep and 20 in cows. Immediately after removal each bag was rinsed with tap water, washed with clear water in a washing machine, dried at 70 EC and weighed. The residues were then pooled per feed, animal and incubation time and finally ground to pass through a 1 mm sieve. These samples were analysed following official Dutch protocols (Nederlands Normalisatie Instituut, 1992) (NEN) comparable to those of the Association of Official Analytical Chemists (AOAC, 1984). The residues were analysed for contents of dry matter (DM, NEN 3332), ash (NEN 3329) and nitrogen (N, Dumas).

Experiments

Different treatments within years were carried out consecutively with the same animals (all animals were given the same treatment during the same period). Each treatment lasted for three weeks to give time for the execution of the *in situ* rumen incubations. An adaptation period of two weeks was allowed between incubation series. In this experimental design treatment and period were confounded and the results may have been influenced by when the treatments were carried out.

Experiment 1
The reference values for Kd and U of DM and CP of the feeds were estimated according to standard ID-DLO procedures including dairy cows. A comparison could then be made between reference values for rumen kinetics and those measured in sheep to show any differences between species and any effects of their different diets.

Experiment 2
Ewes of two different breeds were consecutively fed two diets which differed in concentrate allowance by approximately 150 and 300 g/kg of dietary concentrate DM (coded LC and HC). This was to investigate the influence of the roughage:concentrate ratio of the diet on Kd and U of DM and CP of the feeds under consideration. A comparison was also made between sheep breeds (for treatment HC). The results from the sheep fed the HC treatment were used for the comparison with reference values for rumen kinetics (comparison between species).

Experiment 3
Unshorn and shorn crossbred ewes were fed diets based on energy and protein requirements for unshorn ewes. Approximately 300 g/kg dietary DM originated from concentrates. The mean daily ambient temperature was 3.7 EC (s.d. 3.4). The influence of shearing (mild cold exposure) on Kd and U of DM and CP of the feeds under consideration was investigated for ewes fed at the same feeding level.

Table 2.3
Experiments performed ; incubated feeds, number and type of animals and daily allowances

Experiment 1 : Reference values for Kd and U, standard procedure performed with cows

Year	Incubated feed[†]	Cows	Daily allowance (g dm)
1993	H1 CL1 CH1	n = 3	3610 FMS[‡] +4100 ADG[§]
1994	H2 CL2 CH2	n = 3	3610 FMS[‡] +4100 ADG[§]

Experiment 2 :Kd and U for sheep, comparison of dietary roughage:concentrate ratio (1993a,b ; tested for within animal effect) and of breeds (1993/1994)

Year	Incubated feed[†]	Texel	Crossbred	Daily allowance (g dm)
1993a	H1 CL1 CH1	n = 2[a]	n = 2[b]	1120 hay[l] +180 conc[¶]
1993b	H1 CL1 CH1	n = 2[a]	n = 2[b]	860 hay[l] +355 conc[¶]
1994	H2 CL2 CH2	n = 3	n = 3	980 hay[l] +350 conc[¶]

Experiment 3 :Kd and U for sheep, comparison of unshorn and shorn crossbred ewes (tested for within animal effect for three years)

Year	Incubated feed[†]	Unshorn	Shorn	Daily allowance (g dm)
1994	H2 CL2 CH2	n = 3[c]	n = 3[c]	980 hay[l] +350 conc[¶]
1995	H3 CL3 CH3	n = 4[d]	n = 4[d]	980 hay[l] +350 conc[¶]
1996	H4 CC	n = 4[e]	n = 4[e]	860 hay[l] +360 conc[¶]

[†]H=hay, CL=concentrate low protein, CH=concentrate high protein and CC=commercially available concentrate
[‡]forage maize silage
[§]artificially dried grass
[l]the hay fed was identical to the incubated hay
[¶]concentrate fed, an equal mixture of the incubated concentrates
[a-e] same ewes tested for two ratio's hay:concentrate (a, b) or before and after shearing (c, d, e)

Diets

Daily allowances for each experiment are given in Table 2.3. The cows were fed a mixture of maize silage and artificially dried grass (*Lolium perenne)* twice a day. The ewes were fed according to an average live weight of 70 kg on a feeding level of 1.5 * maintenance

(comparable to late gestation). The sheep diets always consisted of the feeds incubated in the rumen. Roughage and concentrates were offered separately at the same mealtime twice daily. The concentrates fed were either an equal mixture of CL and CH or CC (1996).

Calculations

Feed components are made up of three fractions : the slowly degradable fraction (D), the rumen undegradable fraction (U) and the easily washable fraction (S). The degradation rate (Kd, $\%h^{-1}$) of the slowly degradable fractions of DM and CP was calculated according to a first order model based on Ørskov and McDonald (1979) and adapted by Robinson, Fadel and Tamminga (1986) :

$$Dr_t = U + D * \exp^{\{-Kd*(t-T_0)\}}$$

where : Dr_t = % residual D at t
U = % undegradable residue
D = % slowly degradable fraction
Kd = degradation rate per hour
T_0 = lagtime
t = incubation time

This model was used for residues after *in situ* incubation for 0, 2, 4, 8, 12, 24, 48, 72 and 336 hours and included a test for a lag phase preceding the onset of rumen degradation. The undegradable residue (U) was measured as a residue after *in situ* rumen incubation for 336 hours. The easily washable fraction (S) was measured as the fraction disappearing from the nylon bags during washing without rumen incubation. The slowly degradable fraction (D) was calculated as D = 100 - U - S.
The statistical analysis was performed with the statistical package GENSTAT 5 (Payne, Lane, Ainsley, Bicknell, Digby, Harding, Leech, Simpson, Todd, Verier and White, 1987).
Comparisons of the effect of dietary roughage:concentrate ratio and shearing were made using a within animal test (Students t-test) and thus within feeds and within years (within years these treatments were imposed consecutively on the same ewe).
Comparisons of the effect of breeds and species were made by means of analysis of variance for separate years and over years after including a correction for the experimental year.

Results

Comparison of species and sheep breeds

Table 2.4 contains the average measured Kd and U of DM and CP of the feeds and the results of the statistical analysis.

Table 2.4
Comparison of estimated Kd (% per hour) and measured U (%) between dairy cows reference and ewes and between ewes of different breeds. Significant differences are indicated[¶].

		Kd			Cow v Tex[†]		Cow v cros[‡]		Tex[†] v cros[‡]	
Feed[§]	Comp[|]	Cow	Tex[†]	Cros[‡]	S.e.d.	P	S.e.d.	P	S.e.d.	P
H1,H2	DM	2.1	3.2	1.4	0.32	**	0.14	**	0.36	**
	CP	2.6	3.7	2.1	0.40	*	0.22	*	0.47	*
CL1,CL2	DM	7.2	11.7	9.2	0.74	***	0.75	*	0.83	*
	CP	4.2	10.1	7.9	0.59	***	0.72	***	0.92	*
CH1,CH2	DM	5.5	10.0	7.1	1.09	**	0.82		1.36	
	CP	3.0	7.8	4.2	0.85	***	0.48	*	1.08	*

		U			Cow v Tex[†]		Cow v cros[‡]		Tex[†] v cros[‡]	
Feed[§]	Comp[|]	Cow	Tex[†]	Cros[‡]	S.e.d.	P	S.e.d.	P	S.e.d.	P
H1,H2	DM	19.8	12.8	26.9	2.63	*	3.35		4.10	*
	CP	15.9	9.5	17.4	1.75	**	2.61		3.17	*
CL1,CL2	DM	5.8	4.5	5.7	0.43	*	0.72		0.86	
	CP	4.6	4.6	5.2	0.72		0.95		1.15	
CH1,CH2	DM	4.3	4.3	6.0	0.34		0.54		0.67	*
	CP	0.9	1.0	3.3	0.17		1.18		1.33	

[†]Texel ewes
[‡]crossbred ewes
[§]H=hay, CL=concentrate low protein, CH=concentrate high protein and CC=commercially available concentrate
[|]chemical component, DM = dry matter and CP = crude protein
[¶] * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

The degradation rate was affected by species and sheep breeds. Cows displayed lower Kd's ($P<0.05$) than sheep for concentrates. For hay no clear trend between cows and sheep was observed. However, cows displayed lower Kd's ($P<0.05$) than Texel ewes and higher ($P<0.05$) Kd's than crossbred ewes. The comparison between sheep breeds showed for all feeds that Kd's measured in Texel ewes were higher ($P<0.05$) than for crossbred ewes.

The undegradable fraction of the feeds was not significantly affected by species or sheep breeds. Cows displayed comparable U's to sheep for all feeds, although for hay and concentrate CH (DM) cows had higher (P<0.05) U's than Texel ewes. Cows seemed to have lower U's than crossbred ewes but these differences never reached statistical significance. Texel ewes seemed to display lower undegradable fractions of the feeds than crossbred ewes and significant differences (P<0.05) were found for hay and concentrate CH (DM).

The influence of dietary roughage:concentrate ratio in ewes

The average results (per treatment) for Kd and U of the feeds (DM and CP) and the results of the statistical analysis are given in Table 2.5.

Table 2.5
Comparison of estimated Kd (% per hour) and measured U (%) between diets with different roughage:concentrate ratio.

		Kd				U			
Feed[†]	Comp[‡]	LC[§]	HC[ǀ]	S.e.d.	P	LC[§]	HC[ǀ]	S.e.d.	P
H1	DM	2.0	2.1	0.26	0.96	22.8	19.6	4.23	0.50
	CP	2.8	2.6	0.38	0.74	14.9	13.0	2.94	0.56
CL1	DM	13.9	13.1	1.67	0.65	5.6	3.9	0.84	0.13
	CP	10.1	9.0	1.40	0.50	5.6	4.3	0.91	0.23
CH1	DM	6.5	8.0	0.62	0.10	8.7	5.7	1.37	0.12
	CP	6.2	5.5	1.09	0.57	7.6	3.7	2.77	0.25

[†]H=hay, CL=concentrate low protein and CH=concentrate high protein
[‡]chemical component, DM = dry matter and CP = crude protein
[§]low concentrate diet, 150 g concentrates per kg diet (DM)
[ǀ]high concentrate diet, 300 g concentrates per kg diet (DM)

Table 2.5 shows that Kd and U of the feeds under consideration did not differ for DM and CP between treatments (P>0.05). Further within-breeds analysis showed similar results, although breeds differed in Kd and U (Table 2.4).

The influence of shearing (mild cold exposure)

The average results (per treatment) of Kd and U (DM and CP) of the feeds tested and the results of the statistical analysis are represented in Table 2.6.

Table 2.6
Comparison of average Kd (% per hour) and measured U (%) between shorn and unshorn crossbred ewes. Significant differences are indicated[¶].

		Kd				U			
Feed[†]	Comp[‡]	US[§]	S[|]	S.e.d.	P	US[§]	S[|]	S.e.d.	P
H2-H4	DM	2.3	2.6	0.34		23.5	17.7	1.48	**
	CP	3.1	3.8	0.26	*	14.6	11.6	1.00	*
CL2,CL3	DM	9.2	9.2	0.82		7.1	7.0	0.17	
	CP	8.9	9.4	0.66		5.2	4.8	0.42	
CH2,CH4	DM	8.2	9.3	0.89		5.0	4.3	0.30	
	CP	5.0	6.8	0.64	*	0.9	0.8	0.10	
CC	DM	3.6	5.3	1.77		16.9	15.3	0.40	*
	CP	1.9	3.9	1.38		10.4	8.2	0.37	*

[†]H=hay, CL=concentrate low protein, CH=concentrate high protein and CC=commercial concentrate
[‡]chemical component, DM = dry matter and CP = crude protein
[§]unshorn ewes
[|]shorn ewes
[¶] * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$

Cold exposure as a result of shearing tended to increase degradation rate and decrease the undegradable fraction. The Kd's of the feeds measured in unshorn ewes was lower than in shorn ewes, but significant differences (P<0.05) were only found for hay (CP)and concentrate CH (CP). The observed U of the feeds was higher for unshorn ewes and reached statistical significance (P<0.05) for hay and concentrate CC (DM and CP).

Discussion

Experimental design

In Experiment 3 alternation of treatments was impossible because of the irreversible process of shearing. In Experiment 2 a similar design to Experiment 3 was chosen. Incubation of a control feed was impossible because of a limitation in the number of bags allowed per incubation period in sheep. Therefore, the results may have been influenced by the period in which the treatments were imposed (confoundment of treatment and period effects). Often period effects are not measurable for sheep and cattle (Lindberg, 1985 ; Mehrez and Ørskov,

1977 ; Weakly, Stern and Satter, 1983 ; Ehle, Murphy and Clark, 1982), although they have been reported for cattle by some workers (Figroid, Hale and Theurer, 1972 ; Nocek, 1985). Literature on sheep (Lindberg, 1985 ; Mehrez *et al.*, 1977) or on cows when using rations comparable to the rations fed in our experiments (Weakly *et al.*, 1983) implies an absence of period effects. Therefore a compromise experimental design was used.

Differences between rumen kinetics in dairy cows and in sheep

Our comparison between the results of *in situ* rumen incubations in ewes and in dairy cows (where any effects of species and their diets were considered) demonstrated that reference protein values of feedstuffs should not be used for sheep. This incompatibility is influenced by differences in the rations fed, in *in situ* technique (caused by the smaller rumen size of sheep) and in the amount of nutrients recycling in the rumen (Nocek, 1988 ; Lindberg, 1985). In accordance with results of Siddons and Paradine (1983), who used rations comparable to those fed in our experiments, and of Udèn and van Soest (1984), we found higher degradation rates in sheep. This supports our view that differences between rumen kinetics of ewes and dairy cows were not caused by methodological differences alone, but were also due to between species differences.

Differences between rumen kinetics in Texel ewes and in crossbred ewes

Differences between *in situ* rumen kinetics within species are related to sex, age and physiological state and may be related to the specific diet associated with the physiological state (Nocek, 1988 ; Huntington *et al.*, 1995). Few data are available about differences between sheep breeds. Sheep of the Dorper and Merino breeds displayed statistically significant differences for *in sacco* DM disappearance (De Waal,1995). Our comparison between sheep breeds (comparable in live weight, sex, age, physiological state and diet) showed that Texel ewes had on average 45% (±29) higher Kd's and 35% (±33) lower U's than crossbred ewes. Notably, these breeds showed large differences in Kd's for hay (DM 78% and CP 55%), indicating more cellulolytic fermentation for Texel ewes. This might have been caused by more intense chewing and ruminating facilitating a greater reduction in particle size and increased saliva production. Smaller particle size and a higher pH (in the rumen of Texel ewes) favour fibre degradation (Lindberg, 1985 ; Ørskov and Ryle, 1990). The relatively low degradation rates measured in crossbred ewes (for hay even 20-40 % lower than in cows) were unexpected. This finding may be important in feeding crossbred ewes since sheep diets consist mainly of roughage.

Influence of the dietary roughage:concentrate ratio on rumen kinetics in ewes

In general an relative increase (%) of concentrates in the diet results in a decreased degradation rate of cell walls and protein of roughages (Ganev, Ørskov and Smart, 1979 ; Lindberg, 1981 ; Siddons and Paradine, 1981 ; Weakly *et al.*, 1983 ; Zhao, Shimojo and Goto, 1993 ; De Waal,

1995 ; Archimède, Sauvant, Hervieu, Ternois and Poncet, 1996). However, when CP degradation is not limited by its association with fibrous components (high quality roughages) the influence of roughage:concentrate ratio on CP degradation is not significant (Cronjé,1992). This might explain why during our experiments with high quality hay no effect of roughage:concentrate ratio on Kd and U in sheep was observed.

Influence of cold exposure on rumen kinetics in ewes

The effects of cold exposure (shearing) on rumen digestion kinetics are related to an increase in rumen outflow rate (Ngongoni, Robinson, Kay, Stephenson and Atkinson, 1987) and to an increase of the efficiency in microbial synthesis (Kennedy *et al.*, 1976 and 1977). An increase in the efficiency of microbial synthesis may increase degradation rate, although *in vitro* gas production experiments showed that microbial activity in sheep was not increased by cold exposure (Kennedy, Christopherson and Milligan, 1982). Our results showed that shorn ewes (fed according to energy and protein requirements of unshorn ewes) displayed higher CP degradation rates than unshorn ewes for hay (20%) and concentrate CH (30%). They also displayed smaller undegradable fractions than unshorn ewes for hay (DM 28% and CP 23%) and concentrate CC (DM 10% and CP 24%). This increase in Kd may be an indication of an increase in efficiency of microbial mass synthesis, since *in vitro* gas production and thus the amount of degraded organic matter is unaffected in sheep exposed to cold (Kennedy *et al.*, 1982).

Conclusions

The experiments performed support the view that reference protein values of feedstuffs may not be valid for sheep, since rumen degradation kinetics were different in cows and ewes. There were also differences in degradation kinetics between sheep breeds and between unshorn and shorn ewes. No effect on rumen degradation kinetics could be attributed to dietary roughage:concentrate ratio. In conclusion, it would seem that a different approach to feed protein evaluation is required for cows, sheep, sheep breeds and (un)shorn ewes.

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Chapter 3

Rumen digesta kinetics in cold exposed prolific sheep : impact on protein evaluation

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Abstract

Periodical in vivo measurements of the rumen degraded and undegraded protein fractions were used to study the effects of midwinter shearing of prolific ewes on the effective rumen degradability of dietary protein (EPD). The obtained values were compared to EPD estimated with the standard method of in situ feed characteristics and an assumed rumen passage rate.

The effect of the experimental treatments (unshorn and shorn) was investigated in ewes during a period of three years (n=11). The rumen was emptied and sampled 1, 2, 4, 8 and 12 hours after feeding to monitor rumen contents of dry matter (DM), crude protein-free organic matter (OM-CP) and nitrogen (N). The degraded and undegraded fractions of DM, OM-CP and N in rumen contents were measured by in situ rumen incubation of rumen content samples.

Protein evaluation based on EPD estimated from changes in rumen digesta volume with time displayed more variation and 0.10 more digestible protein in the small intestine (DVE) than the commonly used EPD.

Midwinter shearing of crossbred ewes increased the extent of degradation of DM and OM-CP in the rumen. The extent of degradation of protein was also higher in shorn ewes, but not statistically significant. These differences did not result in different DVE yields upon assumption of an unchanged efficiency of microbial protein growth during cold exposure.

In conclusion the commonly used EPD is more suitable for routine techniques than EPD based on changes in rumen digesta volume with time. However, it should be taken into consideration that the commonly used EPD may result in an underestimation of the DVE value for prolific sheep. Protein evaluation should take account of differences in efficiency of microbial production between unshorn and shorn ewes to avoid additional underestimation of the DVE value.

Keywords: rumen digestion, protein degradation, shearing, cold exposure, ewe

Introduction

Protein evaluation systems for ruminants aim to quantify the amounts of rumen digestible undegraded dietary protein and rumen synthesised microbial protein entering the small intestine. Comparative studies with *in situ* nylon bags (Šebek and Everts, 1999) showed that rumen degradation rate (Kd) and the rumen undegradable fraction (U) of dietary protein differed when determined in unshorn and (winter) shorn prolific ewes. However, these differences in Kd and U may be compensated by different rumen passage rates (Kp).

The *in vivo* estimation of the amounts of dietary and microbial protein entering the small intestine is laborious, expensive and is restricted by small numbers of animals, short duration of experiments, use of markers and complicated chemical analyses to distinguish

dietary protein and microbial protein (Mathers and Miller, 1981 ; Miller, 1982). Therefore, it is widely accepted that the amount of dietary protein entering the small intestine is estimated by the calculation of the amount of rumen (un)degraded feed protein from *in sacco* data. However, the effect of passage of rumen contents is missing in the *in situ* nylon bag method and this leads to overestimation of the actual rumen degradation (Ørskov and McDonald, 1979 ; Lopez et al., 1994). Thus, EPD was introduced combining degradation rate (Kd) and Kp (Ørskov and McDonald, 1979). Passage rate can be estimated by measuring the dilution of an undegradable marker in the rumen and can be expressed proportional to the amount of digesta, which leaves the rumen per hour (Udén et al., 1980). This method, although reliable for fluids, is less reliable for solids (Aitchison et al., 1986b ; Amici et al., 1997) and arbitrary assumptions are needed to model the flow of markers through the rumen (Ramazin, 1995). Therefore, most protein evaluation systems assume constant protein passage rates for roughage and concentrates. This assumption may be questionable when the effects of cold exposure on rumen degradability of feed protein are to be investigated (Kennedy et al., 1986).

The objective of the present experiments was to study the effect of midwinter shearing (mild cold exposure) on the effective rumen degradability of dietary protein in prolific ewes. As a possible alternative to the use of *in vivo* measurement of EPD or the use of Kp, EPD was estimated from changes with time in the rumen volume of protein partitioned into its rumen degraded and rumen undegraded components. The results were compared to the commonly used EPD resulting from a combination of *in situ* determined feed characteristics (Kd, the soluble fraction S and the potentially degradable fraction D) and a characteristic of rumen kinetics (Kp).

Materials and methods

Experiment

The experiment involved rumen emptying to monitor changes with time in rumen content partitioned into its (un)degraded components of nutrients and of rumen microbial mass. The nutrients under consideration were dry matter (DM), crude protein-free organic matter (OM-CP) and nitrogen (N). The experiment was repeated over three years (1994/1996) with 3 animals per treatment in 1994 and 4 animals per treatment in 1995/1996. Treatments (unshorn and shorn) were consecutively applied to the same animals. All animals were given the same treatment during the same period. From results described in literature it was expected that the possible confoundment of treatment and period did not interfere with treatment effects (Šebek and Everts, 1999). Measurements in shorn ewes were performed during the fifth week after shearing at a mean daily ambient temperature of 3.7 (s.d. 3.4) °C.

Animals and diets

The animals used were mature non-pregnant, dry ewes of a prolific crossbred (Ile the France ♂ x Finnish Landrace ♀) with an average live weight of 71 kg (sem = 3.1). The ewes were fitted with a rumen cannula of 6.5 cm internal diameter (Bar Diamond Inc., Idaho, USA) and were kept indoors. In 1996 data from one animal had to be removed due to high feed refusals. The ewes were offered hay (mainly *Lolium perenne*) and concentrates according to energy and protein requirements (CVB, 1992) as if they were ewes in late pregnancy. The daily allowance in 1994/1995 was 980 grams DM of grass hay and 350 grams DM of concentrates and in 1996 it was 860 grams DM of grass hay and 360 grams DM of concentrates. Roughage and concentrates were fed twice daily (at 8.00 h and 20.00 h) and were offered separately but at the same time. Between years the hays differed and the concentrates were of comparable feeding value but differed slightly in composition (Table 3.1).

Table 3.1
Chemical composition of ration components (g/kg DM)

	Hay			Concentrates		
	1994	1995	1996	1994	1995	1996
Dry matter (g/kg product)	894	865	860	872	870	902
Ash	107	100	100	72	70	107
Crude protein	192	141	148	228	235	187
Crude fat	21	23	24	58	62	40
Sugar	na[a]	na[a]	na[a]	78	96	90
Starch	na[a]	na[a]	na[a]	244	246	103
Neutral detergent fibre	602	526	562	145	140	316
IVDOM[b]	0.71	0.74	0.74	0.84	0.84	0.81

[a] not analysed

[b] *in vitro* digestibility of organic matter (Tilley and Terry, 1963)

Rumen emptying

The rumens were emptied at 1, 2, 4, 8 and 12 hours after morning feeding. Feed intake was registered (allowance minus refusals) and the total rumen content of solids and fluids was removed and weighed. After thorough mixing two samples of approximately 100 g DM were taken and the remaining rumen content was replaced into the rumen. The sampling procedure was randomised across time points. To minimise disturbance to rumen

fermentation due to rumen emptying, two meals were fed between emptyings. Thus, the rumen was emptied on five consecutive days. The samples of rumen content were freeze-dried and ground to pass a 3 mm sieve. Samples were analysed for DM, ash, N and diaminopimelic acid (DAPA). The amount of OM-CP was calculated by difference. On the fifth day of rumen emptying, one litre of rumen fluid was also collected to isolate rumen bacteria by differential centrifugation as described by Robinson et al. (1987). Rumen bacteria were analysed for N and DAPA in order to calculate the ratio N to DAPA.

Partitioning of rumen content

The partitioning of rumen content into its degraded and undegradable components of DM, OM-CP and N was based on *in situ* rumen incubation of rumen content samples. In a pilot trial several procedures for sample preparation were compared ; incubation of untreated fresh rumen content and incubation of freeze-dried rumen content. The freeze-dried rumen content was treated in four ways : 1) ground to pass a 1 mm sieve 2) ground to pass a 3 mm sieve 3) chopped to an a average length of 1 cm or 4) untreated. From this pilot trial it was concluded that incubation of freeze-dried rumen content after grinding to pass a 3 mm sieve yielded similar results as incubation of fresh rumen content. Thus freeze-dried rumen content (3 mm) was used in all experiments.
From each emptying (1, 2, 4, 8 and 12 hours after feeding) samples of rumen content were also used to fill three nylon bags. One nylon bag was washed in a washing machine with clear water to estimate the soluble rumen content fraction (S_{rumen}). Two nylon bags were incubated *in situ* for 336 hours (Tamminga et al., 1994) to estimate the rumen contents undegraded component (U_{rumen}). The potentially degradable component of the rumen content (Xd) was calculated as :

$$Xd = 100 - U_{rumen} - S_{rumen}$$

The samples of rumen content were incubated in the same animal from which they were obtained. The incubation procedure was as described by Šebek and Everts (1999). The nylon bags with incubation residues were rinsed with water, washed in a washing machine with clear water and dried at 70°C. The residues were weighed, ground to pass a 1 mm sieve and analysed for DM, ash and N.

Estimation of whole tract apparent digestibility

The whole tract apparent digestibility was estimated using indigestible acid detergent fibre (IADF) as an internal marker. Faeces were collected by grab sampling from the rectum two days after the last rumen emptying. Starting 4 hours after morning feeding samples were taken every hour until at least 750 g of faeces had been collected. Immediately after collection the faecal samples were stored at – 20 °C. Feeds and faeces were analysed for

DM, ash, N and IADF. Feeds were also analysed for neutral detergent fibre (NDF), sugar, starch and *in vitro* digestibility of organic matter.

Chemical analyses of samples

The samples were analysed for DM, ash, N, NDF, starch and sugar contents as described by Vuuren van, et al. (1993a). NDF was assayed without sodium sulphite, with alpha amylase and without residual ash. IADF was analysed as described by Penning and Johnson (1983).The *in vitro* digestibility of organic matter was analysed according to Tilley and Terry (1963) and DAPA was analysed as described by Vuuren van, et al.(1993b).

Calculations

The effective rumen degradability of feed protein was calculated from measured changes with time in rumen protein content partitioned into its degraded and undegraded component. Two different models (1 and 2) were used to calculate EPD. The models were fitted (iteratively with a least squares method) for each animal individually and an over-all within years test was used to detect outlayers (residual standard deviation > 2). EPD calculated with model 1 will be referred to as EPD_{rumen} (%). Model 1 was a dynamic rumen model (as described by Aitchison et al., 1986a) based on feed intake, period of feed intake and (un)degraded rumen contents of protein at several intervals after feeding. Model 1 applies to the behaviour of a specific nutrient fraction of the diet in the rumen, partitioned into its degraded (Xd) and undegraded (Xu) components. It consisted of three equations (1a, 1b and 1c) that were used consecutively. First, equation 1a was used to calculate the fractional rate of passage from the rumen (Kp_{rumen}, h^{-1}) of the undegraded component of the nutrient under consideration. Then, under the assumption that Kp_{rumen} of the undegraded and degraded component are equal, equation 1b was used to calculate the fractional rate of degradation in the rumen (Kd_{rumen}, h^{-1}) of the degraded component of the same nutrient. Finally, equation 1c was used to estimate the extent of effective rumen degradation of the nutrient under consideration.

$$Xu = [Xu(0) + (e^{Kp_{rumen} \times Te} - 1) \times Fu / Kp_{rumen}] \times e^{-Kp_{rumen} \times t} \quad (1a)$$

$$Xd = [Xd(0) + (e^{(Kd_{rumen} + Kp_{rumen}) \times Te} - 1) \times Fd / (Kp_{rumen} + Kd_{rumen})] \times e^{-(Kp_{rumen} + Kd_{rumen}) \times t} \quad (1b)$$

$$EPD_{rumen} = Kd_{rumen} \times (1-f) / (Kd_{rumen} + Kp_{rumen}) \quad (1c)$$

Where Xu(0) = Xu in the rumen at t = 0 (g), Te = observed eating time (h), Fu = intake of rumen undegradable nutrient (g h^{-1}), t = time after feeding (h) and Xd(0) = Xd in the rumen at t = 0, Fd = intake of rumen degradable nutrient (g h^{-1}) and f = proportion of the undegradable component in the quantity ingested at t = 0.

This model was used not only to estimate EPD_{rumen} of protein, but also the effective rumen degradability (ERD_{rumen}) of DM and OM-CP as well as to estimate the rumen degradation rate (Kd_{rumen}) and the rumen passage rate (Kp_{rumen}) of protein, DM and OM-CP.

EPD calculated with model 2 will be referred to as EPD_{exp} (%). Model 2 consisted of two equations (2a and 2b). Equation 2a fitted for Xd the curve of disappearance from the rumen as measured between two feedings. This equation was fitted with relative rumen contents, expressing rumen content at different emptying times as a percentage of the rumen content at 1 hour after the start of feeding. Equation 2b was used to calculate the effective rumen degradability of feed protein. It was also used to estimate K, A and B for DM, OM-CP and N.

$$RC_t = A + B * e^{-K*t} \quad (2a)$$

$$EPD_{exp} = 100 - A \quad (2b)$$

Where RC_t = rumen content of Xd at t = t hours after feeding, A = estimated rumen content of Xd at t = ∞ hour
after feeding, B = estimated disappeared rumen content of Xd at t = ∞ hours after feeding, K = estimated fractional rate of disappearance of Xd from the rumen (h^{-1}) and t = time after feeding (h).

Both EPD_{rumen} and EPD_{exp} were compared to the results of the commonly used estimation method (equation 3) for the effective rumen degradability of protein (EPD, %) using *in situ* determined feed characteristics in a steady state model according to Ørskov and McDonald (1979).

$$EPD = S + D * Kd / (Kd + Kp) \quad (3)$$

Where S = soluble fraction of the ingested feed (%), D = potentially degradable fraction of the ingested feed (%), Kd = fractional rate of degradation in the rumen (h^{-1}) and Kp = fractional rate of passage from the rumen (h^{-1}).

EPD was calculated by this model using the results of Šebek and Everts (1999) for S, D and Kd of the ration fed. The ration Kp was assumed to be constant at 0.04/h. This Kp was derived from research with sheep fed rations of chopped hay of perennial ryegrass supplemented with maize starch at a high feeding level (Aitchison et al., 1986b) which is comparable to the ration and feeding level of the present experiments.

The effect of the different values for the effective rumen degradability of feed protein (EPD_{rumen} , EPD_{exp} and EPD) on protein evaluation was calculated with equation 4 and according to the Dutch protein evaluation system (Tamminga et al., 1994).

$$DVE = DVBE + DVME - DVMFE \quad (4)$$

Where DVE = amount of true protein truly digested in and absorbed from the small intestine, DVBE = amount of digestible undegraded feed protein, DVME = amount of digestible microbial protein, DVMFE = endogenous protein losses resulting from digestion.

The calculation of DVME and DVMFE require the whole tract apparent digestibilities of OM and DM respectively (Tamminga et al., 1994). These were estimated *in vivo* (based on IADF as a marker) to allow for possible differences between treatments. The *in vitro* digestibility of OM (Tilley and Terry, 1963) was used to correct (equations 5a and 5b) the *in vivo* digestibility level, since the use of IADF as a marker usually results in an 0.10 to 0.15 underestimation of whole tract apparent digestibility (Krysl et al., 1988 ; Judkins et al., 1990 ; Huhtanen et al., 1994). This underestimation is due to the fact that IADF faecal recovery is usually below 1.0. Therefore the correction factor based on OM is a recovery correction and thus can also be used for DM and N.

$$X_{correction} = \textit{in vitro} \text{ digestibility of OM / } \textit{in vivo} \text{ digestibility of OM} \quad (5a)$$

where *in vivo* digestibility of OM and *in vitro* digestibility of OM relate to the average values for unshorn ewes. The individual measured *in vivo* digestibility of OM and DM were corrected using equation 5b.

$$\text{Corrected } \textit{in vivo} \text{ digestibility} = \textit{in vivo} \text{ measured digestibility} * X_{correction} \quad (5b)$$

The amount of microbial protein at several time intervals after morning feeding was calculated from the analysed amount of DAPA in rumen content combined with the analysed ratio between N and DAPA in isolated rumen bacteria.

Statistical analysis

The statistical analysis was performed with the statistical package GENSTAT 5 (Payne et al., 1993). Comparisons of effects of midwinter shearing were performed by analysis of variance with blocks defined as ewe within year (Students t-test). The DM-intake per kg metabolic live weight ($W^{0.75}$) was included as covariable. The model used was :

$$y = b_0 + b_1 * \text{block} + b_2 * \text{intake} + b_3 * \text{treatment} + e$$

where intake = DM-intake per kg metabolic live weight, treatment is either unshorn or shorn and e = error component

Results

Rumen content fractions

During the time interval between two feedings, the D-, U- and S-fractions (%) of rumen content were almost constant within animals for each nutrient under consideration. Shorn ewes had higher average D-fractions and lower average U- and S-fractions of DM, N and OM-CP content of the rumen than unshorn ewes (Table 3.2).

Table 3.2
Comparison of average D, U and S fractions[a] of rumen content in unshorn (Ush) and shorn (Sh) Crossbred ewes. Significant differences are indicated[b]

	DM			N			OM-CP[c]		
	Ush	Sh	S.E.D.	Ush	Sh	S.E.D.	Ush	Sh	S.E.D.
D (%)	45.6	49.6	0.8^{**}	44.7	48.4	1.3^{*}	51.5	55.6	0.7^{***}
U (%)	21.4	20.2	0.6^{t}	10.3	9.7	0.4	26.3	24.8	0.6^{*}
S (%)	33.0	30.2	0.9^{*}	45.0	42.0	1.5^{t}	22.1	19.7	0.6^{**}

[a] fraction D = rumen degraded, U = rumen undegraded and S = soluble or washable
[b] $^{t} = P < 0.10$; $^{*} = P < 0.05$; $^{**} = P < 0.01$; $^{***} = P < 0.001$
[c] OM-CP = crude protein-free organic matter

Rumen kinetics

Shorn ewes tended to ingest more food than unshorn ewes (Table 3.3). Nevertheless, after correction for intake level by including DM intake per kg $W^{0.75}$ in the model, shorn crossbred ewes had smaller rumen pool sizes than unshorn crossbred ewes for DM, OM-CP and N. Rumen digesta flow kinetics were similar on both treatments, although shorn ewes had a lower fractional rate of disappearance from the rumen (K) for N than unshorn ewes. Shorn ewes tended to have smaller asymptotic rumen contents (A) for N and thus the rumen degraded protein fractions (B) appeared higher in shorn ewes than in unshorn ewes, but without statistical significance. Degradation rates in shorn ewes were higher for N ($Kd_{in\ sacco}$) and for DM and OM-CP (Kd_{rumen}). Cold exposure by midwinter shearing did not affect Kp_{rumen}, but it increased the extent of rumen degradation (EPD_{rumen}) of DM and OM-CP ($P = 0.06$). The extent of protein degradation tended ($P = 0.13$) to be higher (EPD) or appeared higher in shorn ewes (EPD_{rumen} and EPD_{exp}).

Table 3.3
Comparison of rumen kinetics in unshorn (Ush) and shorn (Sh) Crossbred ewes. All averages are corrected for DM intake except for Intake. Significant differences are indicated[a]

	DM			N			OM-CP		
	Ush	Sh	S.E.D.	Ush	Sh	S.E.D.	Ush	Sh	S.E.D.
Intake (g/day)	1104	1210	47.9^{t}	31.6	34.4	1.2*	801	878	35.9^{t}
Rumen pool size (g)	912	763	37.7**	34.3	28.5	1.6**	587	492	24.3**
Rumen digesta flow kinetics									
K (%/h)	15.9	17.8	4.9	24.2	14.4	2.5*	16.9	16.7	10.1
A (%)	37.4	34.2	10.9	58.4	35.6	10.7^{t}	35.6	22.8	13.0
B (%)	64.2	63.5	14.6	42.3	64.1	11.4	65.8	75.0	13.3
Rates of degradation									
$Kd_{in\ sacco}$ (%/h)	2.7	3.1	0.4	3.2	4.1	0.3**	2.6	2.9	0.4
Kd_{rumen} (%/h)	3.2	5.0	0.5**	3.8	4.9	0.9	3.7	5.4	0.7*
Rate of passage									
Kp_{rumen} (%/h)	4.3	3.9	0.6	3.1	3.0	0.6	4.5	4.0	0.6
Extent of degradation									
ERD^{b}_{rumen} (%)	34.0	47.6	5.5*	49.0	54.0	7.2	35.5	49.0	6.1^{t}
ERD_{exp} (%)	-	-	-	40.9	57.0	11.6	-	-	-
ERD (%)	-	-	-	57.6	59.9	1.4	-	-	-
Whole tract digest. (%)	72.2	71.9	0.8	74.0	72.2	1.3	76.8	75.6	0.8

[a] $^{t} = P < 0.10$; $^{*} = P < 0.05$; $^{**} = P < 0.01$
[b] ERD = effective rumen degradability

Whole tract apparent digestibility

As expected, the average uncorrected *in vivo* OM digestibility was 0.14 lower than the *in vitro* OM digestibility. Shorn crossbred ewes tended to have decreased (Table 3.3) apparent digestibilities of DM ($P = 0.13$) and OM-CP ($P = 0.16$). The results were not affected by body weight or DM intake.

Microbial protein in the rumen

The estimated amount of microbial protein in the rumen (Table 3.4) tended to be less ($0.11 < P < 0.22$) in shorn ewes and was lowest ($P = 0.06$) 12 hours after feeding.

Table 3.4
Estimated amount (grams) of microbial protein in rumen contents of unshorn and shorn ewes at several time intervals after feeding. All averages are corrected for DM intake. Statistically significant differences are indicated[a]

	Unshorn	Shorn	S.E.D.
hours after feeding			
1	128.2	121.8	4.8
2	121.3	108.4	9.4
4	131.0	115.8	8.5
8	101.1	97.8	4.8
12	92.1	80.7	5.2[t]

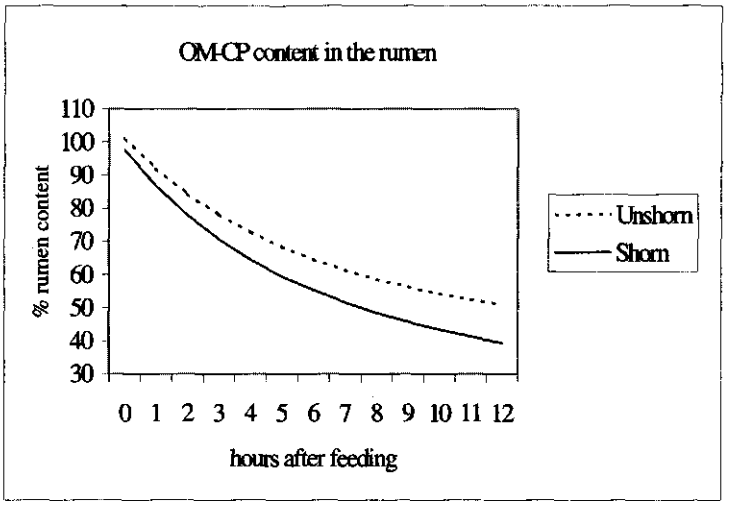
[a] [t] = $P < 0.10$

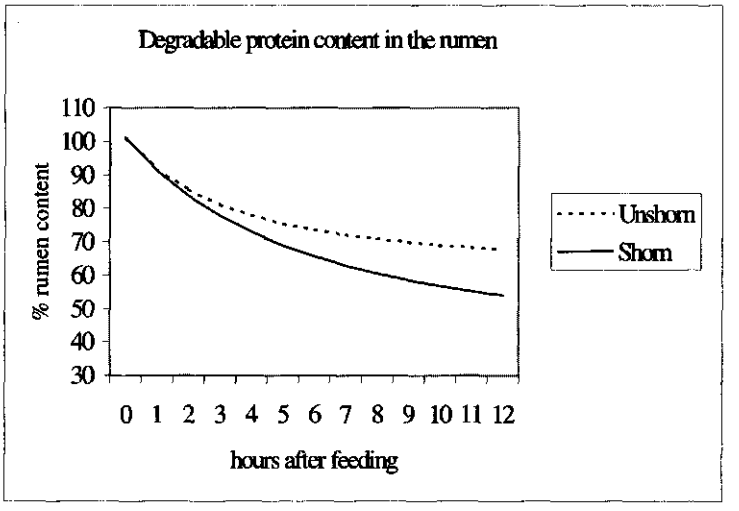
Discussion

Rumen kinetics

The fractional rates of degradation in the rumen were higher in shorn ewes than in unshorn ewes. This should result in larger amounts of degraded nutrients during the period of rumen retention (Ørskov, 1994) when Kp is similar. Indeed ERD_{rumen} of DM and OM-CP showed that shorn ewes degraded larger amounts of nutrients resulting in the observation that shorn ewes had a significantly smaller rumen pool size for DM, OM-CP and N than unshorn ewes. These differences in rumen contents between unshorn and shorn ewes increased during the time interval observed (Figure 3.1). For OM-CP shorn ewes had smaller ($P = 0.06$) rumen contents at 12 hours after feeding and this difference started as a tendency at 10 hours after feeding. A smaller rumen pool size has been associated with a larger ruminal outflow of microbial protein (Chen et al., 1992 ; Meissner et al., 1996 ; Ranilla et al., 1998), with an increased efficiency of microbial growth (Hespell and Bryant, 1979) and with a higher Kp (Chen et al., 1992). A larger ruminal outflow of microbial protein is in agreement with the observed smaller amounts of bacterial N in rumen contents of shorn ewes in the present experiment. Together with an increased efficiency of microbial growth this should lead to an increased amount of microbial protein reaching the small intestine. Indeed, cold exposure increased both the amount of NAN digested in the intestine

Figure 3.1
Estimates for rumen contents (%) of unshorn and shorn ewes (zero hours values = 100%), based on experimental data compiled within this study





(Kennedy et al., 1986) and the efficiency of microbial growth in the rumen of sheep (Kennedy and Milligan, 1978 ; Kennedy et al., 1986).The present experiments show that cold exposure decreases the amount of feed protein that reaches the small intestine. Therefore the amount of microbial protein reaching the small intestine has to increase substantially before there is a significant increase in the amount of protein digested in the small intestine. A higher Kp will support the increased outflow of microbial protein. A higher Kp due to cold exposure (shorn ewes) is reported by Kennedy et al. (1986) and by Ngongoni et al. (1987), but the effects of cold exposure on Kp may be related to the form (chopped or pelleted) in which the diet is given (Kennedy et al., 1982). Other experiments showed that ambient temperature did not alter the retention time of markers of both the particulate and liquid phases of rumen digesta despite significant changes in contraction rate of the reticulum (Kennedy, 1985). In the present experiments cold exposure did not change the observed Kp_{rumen}. Kp_{rumen} concerns the solid phase of the rumen contents of animals fed unchopped grass hay and concentrates and the observed lack in increase of Kp_{rumen} may be in agreement with Kennedy et al. (1982 and 1985). However, the observed Kp_{rumen} may be questionable since it was estimated with a model that was designed to estimate ERD_{rumen}. Aitchison et al. (1986a) tested this model for NDF and concluded that ERD_{rumen} proved to be accurate in predicting the *in vivo* rumen degradability. Aitchison et al. (1986a) also concluded that Kp_{rumen} and Kd_{rumen} were not in agreement with Kp estimated by marker techniques and $Kd_{in\ sacco}$, respectively.

Whole tract apparent digestibility

Whole tract apparent digestibility appeared to be slightly lower in shorn ewes than in unshorn crossbred ewes. This observation is in agreement with Kennedy et al. (1986) who concluded that cold exposure decreased whole tract digestibility in sheep. However, Kennedy et al. (1986) also concluded that the decreased whole tract digestibility due to cold exposure was largely due to a reduction in rumen retention, which seems to be in disagreement with our observation that the extent of rumen degradation increased (Table 3.3).

Implications for feed evaluation of protein

The effects of cold exposure by midwinter shearing were observed in rumen kinetics, whole tract apparent digestibility and presumably in efficiency of microbial protein synthesis. It was discussed that midwinter shearing probably increased the amount of microbial protein reaching the small intestine to a larger extent than that the amount of undegraded feed protein reaching the small intestine was decreased. However, the impact of these changes on the amount of protein digested in and absorbed from the small intestine (DVE) might be low since the intestinal digestibility of microbial protein (approximately 0.64) is much

lower than the intestinal digestibility of undegraded feed protein (approximately 0.85 - 0.90). The Dutch protein evaluation system (Tamminga et al., 1994) was used to investigate the implications of these differences (Table 3.5). The amount of DVE was calculated based on EPD_{rumen} , EPD_{exp} and EPD together with the *in vivo* measured digestible DM and OM.

Table 3.5
Comparison of DVE[a] values (g/kg) based on three different calculations of the effective rumen degradability of feed protein (EPD_{rumen} , EPD and EPD_{exp}). All averages are corrected for DM intake. Statistically significant differences are indicated[b]

	DVE			Significance[c]			
	EPD_{rumen}	EPD	EPD_{exp}	1-2	1-3	2-3	S.E.D.
Differences between unshorn and shorn ewes							
Unshorn	135	122	154		**	***	6.7
Shorn	124	116	118				11.9
S.E.D.	14.3	3.4	21.3				

[a] DVE = true protein truly digested in the small intestine

[b] ** = $P < 0.01$; *** = $P < 0.001$

[c] 1-2 = EPD_{rumen} versus EPD ; 1-3 = EPD_{rumen} versus EPD_{exp} ; 2-3 = EPD versus EPD_{exp}

The use of rumen characteristics to estimate the effective rumen degradability of dietary protein (EPD_{rumen} and EPD_{exp}) resulted in larger variation in calculated DVE values than the use of feed characteristics (EPD). The use of EPD_{exp} resulted for unshorn ewes in higher DVE values than the use of EPD_{rumen} and EPD.

Unshorn crossbred ewes did not differ in DVE yield from shorn ewes, but DVE values tended ($P = 0.13$ for EPD) to be higher in unshorn ewes. However, it is expected that cold exposure increases the amount of NAN digested in the intestine (Kennedy et al., 1986) and thus increases DVE yield. It is reasonable to assume that shorn ewes had a higher efficiency of microbial protein synthesis than unshorn ewes. The present experiments did not provide information on the actual efficiency of microbial protein synthesis. Based on Kennedy and Milligan (1978) who reported increases of 0.13 - 0.42 in cold exposed sheep and the measured 0.49 effective degradability of OM-CP in shorn ewes, the underestimation of microbial protein yield in shorn ewes could be between 0.06 and 0.20. After inclusion of an assumed average efficiency increase for microbial protein synthesis of 0.27 in shorn ewes, DVE yield was 0.10 higher in shorn ewes. This difference will increase further upon inclusion of a higher rumen passage rate in shorn ewes. The use of EPD_{exp} resulted in unexpectedly high DVE values for unshorn ewes. This led (even after inclusion of a higher

microbial protein yield) to a lower DVE yield in shorn ewes than in unshorn ewes. Therefore, DVE values based on EPD_{exp} do not seem to be suitable for estimation of the protein feeding value for sheep. It must be concluded that the use of EPD_{exp} as an estimate for effective rumen degradability of dietary protein is invalid.

Conclusions

The use of models that apply to the behaviour of dietary protein in the rumen to estimate EPD (EPD_{rumen} and EPD_{exp}) was compared to the use of the commonly used EPD. EPD_{exp} appeared to be invalid as an estimate for the effective rumen degradability of dietary protein. EPD_{rumen} examines rumen kinetics in more detail than EPD, but showed more variation and is time consuming to measure. In addition, EPD_{rumen} is based on rations as a whole while feed evaluation usually refers to the additional feeding value of individual feedstuffs. Therefore, EPD is more suitable for routine techniques.
The results showed that midwinter shearing increased the amount of microbial protein reaching the small intestine and at the same time decreased the amount of undegraded feed protein reaching the small intestine. However, the impact of midwinter shearing on the amount of DVE was not statistically significant due to contradicting effects on undegraded feed protein and microbial protein, to differences in intestinal digestibility of undegraded feed protein and microbial protein and to experimental restrictions (large variation and low numbers of animals). The number of animals was restricted by the laborious procedures and the large variation seems to be inherent to the kind of research. Nevertheless, DVE calculated based on EPD was approximately 0.10 lower than DVE calculated based on EPD_{rumen}. Furthermore, DVE values for shorn ewes may be 0.10 underestimated by the current protein evaluation system due to differences in efficiency of microbial protein synthesis. These conclusions imply that in practical feeding evaluation DVE values for unshorn ewes may be underestimated by 0.10 and for shorn ewes by 0.10 to 0.20.

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Chapter 4

Efficiency of nitrogen utilisation during late gestation in prolific ewes

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Abstract

Further consideration should be given to protein utilisation when feeding prolific ewes during late gestation. Protein utilisation in pregnant ewes and protein utilisation for foetal growth were studied using nitrogen (N) balances of pregnant ewes and chemical analyses of new born lambs.

During 4 years (1992/1995) 46 N-balance measurements were performed on pregnant prolific ewes (average litter size > 3) at gestation stage day 115 to 125 (n=36) or during day 130 to 140 (n=10). The animals were selected from two feeding trials with treatments that induced different levels of apparently digestible N (ADN) intake. From the same group of ewes 48 male lambs (stillborn or euthanised immediately after birth) were used to determine body content of dry matter, ash, protein, fat and gross energy.

The N-retention in pregnant ewes and the urinary N output increased with increasing ADN intake. Concomitantly N-mobilisation from the maternal body decreased. As a result the N-accretion in foetal growth was not affected by ADN intake. The chemical composition of lambs from unshorn ewes displayed increasing DM and protein contents as ADN intake increased.

Tis study showed that N from ADN was retained with an overall efficiency of 0.66 in highly prolific ewes in late gestation. The maintenance requirement of these ewes was 444 mg ADN per kg $W^{0.75}$. In ewes with a small positive or a negative N accretion, ADN was used with an efficiency of 0.54 for foetal growth and 0.91 for maternal mobilisation. It was concluded that current feeding strategy is applicable for highly prolific ewes with regard to protein utilisation efficiency in late gestation.

Key words : Nitrogen balance / utilisation / efficiency / foetal N accretion / maternal N change

Introduction

The introduction of prolific sheep breeds (average litter size > 3) is challenging the current feeding strategies for ewes. When fed according to these strategies prolific ewes can have problems with their energy and protein supply during late gestation, causing an increased incidence of acetonaemia and decreased lamb birth weights (Everts, 1990a ; Everts, 1990b). Decreased lamb birth weight influences lamb viability and lamb mortality (Wallace et al, 1996) and decreases postnatal growth and productivity (Bell, 1992). Lamb birth weight is positively related to protein intake (Everts, 1990b), especially when energy intake is low (Quirke et al, 1978). Current requirements are based on research with common breeds and aim to meet requirements of sheep bearing 1 or 2 lambs. Energy requirements have already been investigated by Everts (1990a and 1990b). Therefore, further investigation of the protein requirements for prolific ewes in late pregnancy is essential. The protein

requirement of the pregnant ewe can be presented as the sum of the requirements for maintenance and foetal growth.

The amount of protein used for maintenance during late gestation can be estimated from nitrogen (N) balance studies (Robinson and Forbes, 1966 and 1967 ; McClelland and Forbes, 1971 ; Christenson and Prior, 1976 ; Ngongoni et al., 1989) by extrapolation to zero N retention of the relationship between N retained and digestible N ingested. In these studies maintenance requirements ranged from 0.07 to 0.37 grams digestible N per day per kilogram metabolic weight ($W^{0.75}$) and depended on the level of energy and protein supplied as well as on stage of gestation.

The amount of protein retained in foetal growth can be estimated by analysing the N content of foetuses at various times during gestation (Rattray et al., 1974 ; McDonald et al., 1979 ; Vilette and Thériez, 1984). Protein accretion in foetal growth during gestation depends on energy and protein intake, genotype, litter size and stage of gestation. Equations to estimate N accretion in conceptus growth during various stages of gestation were provided by McDonald et al. (1979).

The efficiency of the utilisation of digestible N for maintenance is assumed to be 0.67 (NRC, 1985). This efficiency can be derived from the relationship between N retained and digestible N ingested. For the efficiency of the conversion of digestible N into N accretion in pregnant ewes literature covers a large range of 0.45 to 0.80 (Everts, 1992). The efficiency of utilisation of digestible N for conceptus growth is assumed to be 0.50 (NRC, 1985). Ngongoni et al. (1989) provide an experimentally determined value of 0.48 for the efficiency of utilisation of truly digested NAN for the net accretion of N in conceptus. However, no separate experimental data is available for estimation of efficiency of N utilisation for maternal maintenance and growth of concepta.

The present study aimed to locate the position of prolific crossbred ewes in the wide range of requirements and efficiencies of protein utilisation described previously. It attemps to determine the amount of protein required for maintenance, the amount of protein accretion in foetal growth and the efficiency of protein utilisation for maternal gain and for foetal growth in prolific ewes during late gestation. This study combined N balance measurements during late pregnancy with chemical analysis of newly born lambs.

Materials and methods

Treatments

Animals were selected from two feeding trials, replicated over two years, to enable measurement of nitrogen (N) balance in late pregnancy. In the first feeding trial protein

intake levels were 0.8, 1.0 and 1.2 times requirement (CVB, 1992) while the diet offered supplied energy according to requirements (CVB, 1992). The effect of winter shearing in late gestation was investigated during the second feeding trial. This trial involved a control group of unshorn ewes and two groups of shorn ewes. The control group and one group of shorn ewes were fed according to energy and protein requirements, whereas the other group of shorn ewes received concentrates at a level comparable to the other two groups but with *ad libitum* grass hay. Treatments in both feeding trials resulted in different allowances of apparently digestible protein.

Animals

Each year, eighty multiparous ewes of a prolific crossbred (Ile the France ♂ x Finnish Landrace ♀) were synchronised in oestrus and mated with Texel rams. The day of mating was counted as day 0 of gestation. The animals were kept on pasture until day 85 of gestation. Thereafter they were housed indoors. At day 95 of gestation the animals were x-rayed to estimate litter size. At day 102 of gestation 60 ewes were selected and individually penned to start a feeding trial. From these 60 animals 12 ewes with an expected litter size of 3 lambs were selected for N balance trials. In total 46 N balance trials were completed with ewes of an average live weight of 81 ± 8.5 kg. After the balance trial the animals were returned into the feeding trial.

Diets

During N balance measurements the ewes were fed 1 kg of grass hay (mainly *Lolium perenne*) daily supplemented with concentrates according to energy requirements based on their expected litter size and their individual live weight at the start of the balance trial. Protein was fed according to treatment and protein levels were established by feeding a mixture of two isocaloric concentrates. One with a low protein content (CL) and another with a high protein content (CH). The experimental diets were offered from at least 10 days before the start of the balance trial. The animals had free access to fresh water. Roughage and concentrates (Table 4.1) were fed twice daily (at 7.00 h and 15.00 h) and were offered separately but at the same time.

N balance

The ewes were housed individually in metabolism crates to enable separate collection of urine and faeces. Wool and dermal losses were collected and analysed together with faeces. Since the animals were already adapted to the diets fed, N balance trials consisted of only 3 days adjustment followed by 10 days of quantitative collection of faeces and urine. In the first experimental year N balance was measured from day 130 to 140 of gestation. Because

Table 4.1
Chemical composition (g/kg DM) of the feeds used

	H†				CL†				CH†			
	1992	1993	1994	1995	1992	1993	1994	1995	1992	1993	1994	1995
Dry matter (g/kg product)	888	860	894	865	878	888	862	863	871	881	881	876
Ash	104	101	107	100	53	56	67	68	58	66	77	73
Crude protein		184	182	192	141	73	70	102	118	301	366	355
Crude fat	35	28	21	23	42	30	58	65	39	48	58	60
Crude fibre	259	285	296	274	54	162	82	88	47	67	58	62
Sugar	na§	na	na	na	112	70	66	90	75	69	91	103
Starch	na	na	na	na	309	312	344	345	261	137	143	147
Neutral detergent fibre	na	562	602	526	143	259	166	156	136	168	124	125
dOM‡	0.73	0.71	0.71	0.74	0.87	0.88	0.83	0.84	0.85	0.85	0.84	0.85

† H=hay, CL=concentrate low protein and CH=concentrate high protein
‡ digestibility of organic matter (Tilley and Terry, 1963)
§ na = not analysed

a number of ewes had large feed refusals attributed to stage of gestation, it was decided to measure N balance from day 115 to 125 of gestation in the following experimental years.

Foetal protein accretion

Chemical analysis was performed on 48 ram lambs with a birth weight of at least 3 kg. The lambs were born from ewes with an expected and observed litter size of 3 and were randomised over treatments. They originated from ewes in the balance trials as well as from ewes in the feeding trials. The lambs used for chemical analysis died during birth or were euthanized immediately after birth (prior to suckling). In each year two sets of at least two lambs were formed per treatment. The average chemical composition of these two sets per treatment was used for every lamb within that treatment. Total foetal N accretion until birth was calculated by multiplying the individual birth weight with the average N content per treatment. The calculation of N accretion during N balance measurement was based on a Gompertz equation for N accretion of foetusus during the prenatal period (McDonald et al., 1979) :

$$\ln y = A - B * \exp(-C * t) + D * (0.00079 * (3 - f) * t)$$

Where y = kg crude protein per foetus, t = days from conception, f = number of foetuses and A, B, C and D = equation constants as provided by McDonald et al. (1979).

The equation constants have been interpreted (Robinson et al, 1977) in terms of weight (A and B) and specific growth rate (C) and parameter D indicates the relationship between the composition of the foetus and its weight at any fixed age. Genotype related differences in N accretion were accounted for by adapting constant B of the Gompertz curve to the analysed N content at birth (including the actual t and f). This adapted equation was used to estimate total foetal N at the start and at the end of N balance measurement. Total foetal N accretion during N balance was calculated as the difference between total foetal N at the end and at the start of the N balance trial.

Maternal N change

For each ewe the difference between retained N and N accretion in foetuses and placenta was taken as an estimate of changes in the N content of the maternal body. N accretion in the placenta was calculated according to a Gompertz curve of McDonald et al. (1979) in which the actual days from conception and number of foetuses were used. Total N accretion in the placenta during N balance was calculated in the same way as total foetal N accretion during N balance. N accretion in foetal fluids was neglected since it was estimated with large variation at 0.12 N in 10 days. Besides, McDonald et al. (1979) reported that the

change in foetal fluids weight was negligible in the period from about 90 to 130 days of gestation.

Chemical analyses of samples

Samples were taken from feeds, faeces, urine and newly born lambs. The samples were analysed following official Dutch protocols (NEN, Nederlands Normalisatie Instituut, 1992), most of which are comparable with those of the Association of Official Analytical Chemists (AOAC, 1984). The analyses performed were dry matter (DM, NEN 3332), ash (NEN 3329), nitrogen (N, NEN 3145), crude fat (Publikatieblad EEG, 1984) and gross energy (GE, NEN-ISO 1928).

Statistical analysis

The statistical analysis was performed with the statistical package GENSTAT 5 (Payne et al., 1987). The model used for fitting the relationship between N retained and apparently digestible N (ADN) intake was :

$$Nretained = b_0 + b_1 * ADN\ intake + e$$

Where b_0 = intercept, b_1 = regression coefficient and e = residual variance

The model used to fit the relationship between ADN intake and foetal N accretion (Nfoe) and maternal N change (Nmat) was :

$$ADN\ intake = b_0 + b_1 * Nfoe + b_2 * Nmat + e$$

Where b_0 = intercept, b_1 and b_2 = regression coefficients and e = residual variance.

Results

N-retention and urinary N output increased with increasing ADN intake (Table 4.2). Concomitantly, N-mobilisation from the maternal body decreased. As a result the N-accretion in foetal growth was not affected by ADN intake. The DM and protein content of lambs in unshorn ewes increased with increasing ADN intake (Table 4.3).

Table 4.2
Results N balances (g / kg $W^{0.75}$ / day) with standard deviation (SD) per treatment and per feeding trial

Treatment[†]	Trial 1			Trial 2		
	1	2	3	2	4	5
Number of ewes (n)	7	7	8	8	8	8
Metabolic weight	27.4 (2.6)	27.1 (1.9)	26.9 (2.6)	27.2 (2.4)	26.3 (2.2)	27.2 (1.2)
Litter size (n)	3.1 (0.4)	2.9 (0.4)	3.4 (0.5)	3.1 (0.6)	3.4 (0.5)	3.5 (0.8)
DM intake	34.4 (13.9)	36.6 (12.7)	33.0 (11.6)	37.7 (11.2)	44.2 (5.6)	51.3 (6.3)
N intake	0.84 (0.30)	0.99 (0.37)	1.10 (0.47)	1.08 (0.35)	1.25 (0.22)	1.40 (0.20)
ADN[‡] intake	0.48 (0.20)	0.62 (0.28)	0.78 (0.34)	0.68 (0.26)	0.80 (0.16)	0.87 (0.14)
ME intake (MJ)	0.39 (0.16	0.42 (0.14)	0.38 (0.12)	0.42 (0.13)	0.49 (0.06)	0.56 (0.07)
N faeces	0.36 (0.12)	0.37 (0.13)	0.33 (0.14)	0.40 (0.10)	0.46 (0.10)	0.53 (0.07)
N urine	0.42 (0.07)	0.52 (0.07)	0.60 (0.21)	0.50 (0.06)	0.56 (0.16)	0.60 (0.12)
N retained	0.06 (0.19)	0.10 (0.23)	0.18 (0.28)	0.18 (0.22)	0.24 (0.11)	0.27 (0.11)
N in conceptus[§]	0.18 (0.04)	0.19 (0.04)	0.20 (0.03)	0.18 (0.03)	0.20 (0.03)	0.20 (0.02)
N change ewe	-0.12 (0.21)	-0.09 (0.25)	-0.03 (0.28)	0.00 (0.22)	0.04 (0.10)	0.07 (0.12)

[†] Treatment 1, 2 and 3 = respectively 0.8, 1.0 and 1.2 times protein requirements (CVB, 1992),
4 = shorn and according to protein requirements and 5 = shorn and *ad libitum* grass hay
[‡] Apparently digested N
[§] accretion of N in foetuses and placenta

Table 4.3
Chemical composition of male lambs at birth (g/kg product and GE† in MJ/kg product) standard deviation (SD) per treatment and per feeding trial.

	Trial 1			Trial 2		
Treatment‡	1	2	3	2	4	5
DM†	212.5 (5.6)	220.5 (4.1)	225.7 (5.1)	223.0 (5.5)	213.6 (1.8)	216.3 (6.2)
Ash	38.8 (1.4)	37.8 (2.3)	41.4 (1.1)	40.3 (2.0)	37.5 (0.4)	38.0 (2.0)
Protein	145.1 (7.4)	155.4 (1.3)	158.7 (5.4)	155.3 (4.3)	150.0 (1.2)	153.9 (6.2)
Fat	21.1 (2.2)	21.1 (1.5)	20.9 (2.1)	22.8 (2.6)	21.1 (1.2)	20.6 (1.4)
GE†	4.5 (0.2)	4.7 (0.1)	4.7 (0.2)	4.6 (0.1)	4.4 (0.1)	4.5 (0.1)

† GE = Gross Energy, DM = Dry matter

‡ Treatment 1, 2 and 3 = respectively 0.8, 1.0 and 1.2 times protein requirements (CVB, 1992), 4 = shorn and according to energy and protein requirements and 5 = shorn and *ad libitum* grass hay

The regression analysis on the relationship between retained N and ADN intake per kg metabolic live weight ($W^{0.75}$) accounted for 78% of the observed variance (adj.R^2) and showed a residual standard deviation (s.d.) of 93. This regression resulted in (Nretained and ADN intake in mg per kg $W^{0.75}$ per day) :

Nretained = -293 (41) + 0.660 (0.05) ADN intake

From this equation the endogenous urinary N loss and the amount of ADN required for maintenance of N balance in the ewes were estimated to be 293 and 444 mg N / kg $W^{0.75}$ / day respectively. Linear regression revealed that the efficiency of utilisation of ADN in late gestation was 0.66 (Figure 4.1). Figure 4.1 indicates possible differences between ewes with a positive and negative N balance in the relationship between retained N and ADN intake. Inclusion of a qualitative variable for positive or negative N balance (adj.R^2 = 0.83 and residual s.d. = 81) resulted in (Nretained and ADN intake in mg / kg $W^{0.75}$ / day) :

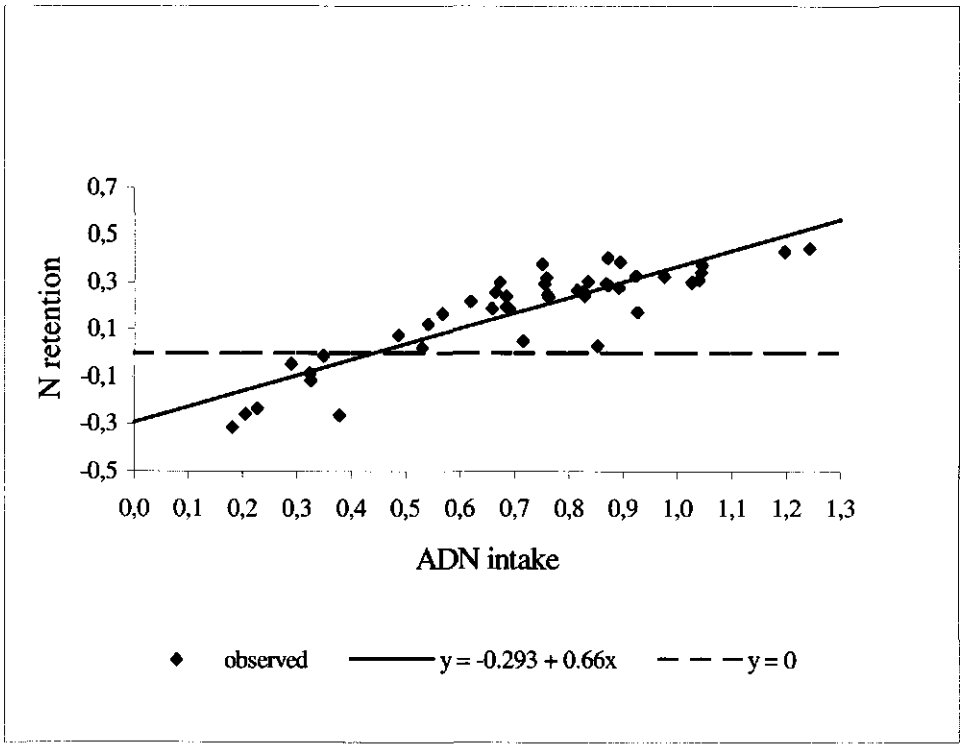
$$\text{Nretained} = \begin{cases} -82\ (64) + 0.417\ (0.08)\ \text{ADN intake} & ;\ \text{for ewes with a positive N balance} \\ -425\ (124) + 0.908\ (0.42)\ \text{ADN intake} & ;\ \text{for ewes with a negative N balance} \end{cases}$$

From these equations the endogenous urinary N loss and the amount of N required to maintain N balance in the ewes in positive N balance were estimated to be 82 and 425 mg N / kg $W^{0.75}$ / day respectively, for those in negative N balance these figures were 197 and 468 mg N. The efficiency of utilisation of ADN in prolific ewes during late gestation was 0.42 and 0.91.

The estimated efficiencies of ADN utilisation may also have been influenced by other factors such as experimental year, period of gestation (Christenson and Prior, 1976) or ME intake (Ngongoni et al., 1989). Additional regression analysis showed that these factors were aliased with the qualitative variable for the animal's N balance (positive or negative) and that they did not contribute to a further explanation of the observed variance.

The efficiencies for the utilisation of ADN for conceptus growth (Nfoe : foetuses and placental tissue) and for maternal tissue N (Nmat) were estimated by multiple regression analyses. Experimental year, stage of gestation, positive or negative N balance, positive or negative maternal N change and ME intake were included as qualitative variables. It was concluded that stage of gestation was the only qualitative variable (including the interactions with Nfoe and Nmat) that contributed significantly to the explanation of the observed variance.

Figure 4.1
Relationship between N retention and ADN intake (g/kg$^{0.75}$/day)



This regression (ADN intake, Nfoe and Nmat in mg / kg $W^{0.75}$/ day adj.R^2 = 0.78 and residual s.d. = 122) resulted in :

$$\text{ADN intake} = \begin{cases} 440\ (123) + 1.56\ (0.66)\ \text{Nfoe} + 1.22\ (0.15)\ \text{Nmat} & ; \text{ around day 120 of gestation} \\ 1296\ (614) - 2.79\ (2.65)\ \text{Nfoe} + 1.02\ (0.25)\ \text{Nmat} & ; \text{ around day 135 of gestation} \end{cases}$$

The results concerning the period around day 135 of gestation showed unrealistic values for maintenance and efficiency of ADN utilisation for conceptus growth. It was therefore concluded that the available data were insufficient for this purpose. For the period around day 120 of gestation, the amount of N required to maintain N balance in the ewes was estimated to be 440 mg N / kg $W^{0.75}$ / day. The efficiencies of utilisation of ADN for conceptus growth and for maternal tissue N were 0.64 and 0.82 respectively around day 120 of gestation.

Discussion

The present study focussed on the apparently digested N (ADN) because of the use of N balance experiments in which ADN was directly measured. The use of ADN was preferred above the use of the Dutch protein evaluation system (Tamminga et al, 1994), which estimates the amount of true protein truly digested in and absorbed from the small intestine (DVE). The reasons for this choice were that this protein evaluation system was designed for dairy cows and its accuracy for sheep may be questioned (Šebek and Everts, 1999). Therefore DVE was thought to be unsuitable for interpretation of the results of N balance measurements. However, the amount of NAN truly digested in and absorbed from the small intestine can be calculated from the measured amount of faecal N and the average apparent digestibility of NAN entering the small intestine in sheep. Literature provides an apparent digestibility of NAN entering the small intestine in sheep within a range of 54% to 72% with an average of 64.4% (Egan and Ulyatt, 1980 ; Merchen et al., 1986 ; Siddons et al., 1984 and 1985 ; MacRae et al., 1985 ; Kelly and Christopherson, 1989 ; Faichney et al., 1997). The average apparent digestibility of NAN entering the small intestine in sheep for diets comparable to the diets in the present experiments was 64.5%. This average was used to estimate the amount of NAN truly digested in and absorbed from the small intestine in the present experiments. The regression analysis (adj.R^2=0.48 and residual s.d.= 144) of the relationship between retained N and the estimated truly digested NAN in mg per kg $W^{0.75}$ per day resulted in :

Nretained = -336 (91) + 0.657 (0.13) truly digested NAN

The results of this regression analysis for the overall efficiency of N utilisation during late gestation was comparable to the results of the regression analysis with ADN intake.

Therefore, it was concluded that the use of ADN was acceptable for estimating the efficiency of N retention in prolific sheep during late gestation

In the present experiment the overall efficiency of the utilisation of ADN during pregnancy was estimated to be 0.66 ± 0.05 in multiparous Finn cross ewes. This result is in agreement with results from other experiments described in literature. The overall efficiency of N utilisation for multiparous Finn cross ewes with a positive N balance at day 135 of gestation was 0.66 and 0.50 at energy allowances of 0.44 and 0.61 MJ per kg $W^{0.75}$ (Ngongoni et al., 1989) and for twin bearing Dorset ewes fed to maintain maternal tissue N it was 0.7 (McNeill et al., 1997). In non pregnant ewes it was 0.58 (Robinson and Forbes, 1966) and in yearling Finn cross ewes it was 0.68 and 0.75 at respectively day 115 and day 135 of gestation (Christenson and Prior, 1976).

The efficiency of the utilisation of ADN differed between ewes with a positive and a negative N balance. The s.e. of the estimated parameters of the latter two relationships was larger than those of the overall relationship and the estimated efficiency (0.42 ± 0.08) for animals with a positive N balance was low compared to Ngongoni et al. (1989). For animals with a negative N balance no other experimentally determined values for prolific ewes were available. In literature (Robinson and Forbes, 1966 ; Christenson and Prior, 1976) data of ewes with a positive and negative N balance were always analysed together. The low efficiency and low maintenance requirement of the animals with positive N balances in the present experiment may be due to a relative large group of animals with ADN intake in excess of requirements (> 0.9 g ADN per kg $W^{0.75}$, AFRC, 1995 ; NRC, 1985). The relationship between retained N and protein intake looses linearity when N intake is in excess of the amount required (McClelland and Forbes, 1971 ; Black and Griffiths, 1975 ; McNeill et al., 1997). Figure 4.1 indicates a tendency to non-linearity for ADN intakes above 0.85 g per kg $W^{0.75}$, but statistical evidence for a non-linear relationship is lacking. Restriction of the dataset to animals with a daily ADN intake of 0.85 g per kg $W^{0.75}$ or less and a positive N balance resulted in the following relationship (Nretained and ADN intake in mg / kg $W^{0.75}$ / day, $R^2 = 0.45$ and residual s.d. = 67):

Nretained = -208 (103) + 0.611 (0.15) ADN intake

The R^2 of this relationship is low, standard errors are relatively large and the estimated efficiency of the utilisation of ADN during pregnancy is within the range (0.66 ± 0.05) of the overall estimated efficiency. Therefore, it was concluded that the overall efficiency of 0.66 on the combined dataset is valid for Finn cross ewes during pregnancy when fed according to protein and energy requirements.

According to the overall relationship between N retention and ADN intake, the amount of ADN necessary to maintain zero N balance was 444 mg per kg $W^{0.75}$. This is in agreement

with the 440 mg per kg $W^{0.75}$ according to the relationship between ADN intake and N for conceptus growth and maternal tissue. It is also in agreement with the 438 mg per kg $W^{0.75}$ for *multiparous* Finn cross ewes at day 130 of gestation (Ngongoni et al., 1989) and with the 273 and 371 mg per kg $W^{0.75}$ for yearling Finn cross ewes at day 115 and 135 of gestation (Christenson and Prior, 1976). Therefore, it was concluded that the amount of ADN necessary to maintain zero N balance is 444 mg per kg $W^{0.75}$ for *multiparous* Finn cross ewes during late pregnancy.

Regression analysis was also used to estimate the efficiencies for the utilisation of ADN for conceptus growth and for maternal tissue N. Stage of gestation contributed to the explanation of the observed variance, but the relatively limited data (n=10 ewes) around day 135 of gestation did not provide a valid relationship. During this period large feed refusals, negative N retention and high levels of maternal N mobilisation were observed. Therefore, it was concluded that this part of the dataset was not valid for this purpose and that further calculations were essential to the investigation of the influence of maternal N mobilisation. This resulted in the following relationship for ewes around 120 days into gestation allowing for inclusion of a qualitative variable for negative or positive changes in maternal N (ADN intake, Nfoe and Nmat in mg per kg $W^{0.75}$, $R^2 = 0.64$ and residual s.d. = 132):

$$\text{ADN intake} = \begin{cases} 387\ (285) + 1.86\ (1.32)\ \text{Nfoe} + 1.10\ (0.41)\ \text{Nmat} & ;\ \text{maternal N change} < 0 \\ 384\ (172) + 1.36\ (0.89)\ \text{Nfoe} + 1.92\ (0.46)\ \text{Nmat} & ;\ \text{maternal N change} > 0 \end{cases}$$

From this equation the amount of N required to maintain N balance was estimated to be 386 mg N / kg $W^{0.75}$ / day for ewes at about 120 days in gestation. The efficiency of ADN utilisation for conceptus growth was 0.54 and 0.74 and for maternal tissue 0.91 and 0.52 respectively in ewes with or without N-mobilisation. It must be emphasised that these estimates have a large s.e., but for ewes with N-mobilisation the efficiency of ADN utilisation for conceptus growth of 0.54 agrees favourably with the 0.48 reported by Ngongoni et al. (1989). However, other experimental data on this subject are not available. For ewes fed according to requirements maternal N changes should be small, but a small positive overall N retention in ewes can hide a substantial net loss of carcass N that is compensated by retention of N in visceral organs and the mammary gland (McNeill et al., 1997). Growth of visceral organs, mammary gland, foetuses and wool are partly dependent on internal N-mobilisation even at N equilibrium of the ewe. Therefore, the relationship for maternal N accretion < 0 was preferred and it was concluded that the efficiency of ADN utilisation was 0.54 for conceptus growth and 0.91 for maternal mobilisation. For prolific ewes in late gestation, when N accretion in maternal tissue is low, this is in agreement with the observed overall efficiency of 0.66 for ADN utilisation.

Conclusions

The results of the experiments described imply that the assumed (in current feeding systems) efficiencies for ADN utilisation in late gestation and for conceptus growth are applicable to prolific crossbred ewes. Combination of these efficiencies with results from protein content analyses in newly born lambs allows estimation of the protein requirement for foetal growth in prolific ewes. However, further attention should be given to the provision of adequate feeding strategies for prolific crossbred ewes.

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Chapter 5

Feed intake, protein requirements and nutrient utilisation of prolific ewes during late gestation

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Abstract

The effects of protein feeding strategy and winter shearing during late pregnancy on feed intake and reproduction performance of prolific ewes were investigated in two experiments. Each experiment involved two years with 60 crossbred ewes. In total 240 ewes were used in the period from 105 days of gestation until lambing. They were fed a concentrate / roughage diet. During the first experiment unshorn ewes were fed at protein feeding levels of 0.8, 1.0 (control treatment) and 1.2 of requirements and energy supply was fed according to requirements. The second experiment involved the same control treatment with unshorn ewes and two groups of shorn ewes. One group of shorn ewes was fed according to energy and protein requirements and the other group was offered grass hay ad libitum. Measurements included feed intake, sum of birth weight of lambs within a litter (SBW), body weight change (BWC), body condition score change (BCSC) and wool growth of the unshorn ewes. Daily dry matter intake was stable in unshorn ewes during late gestation and the average energy ingested (0.45 MJ ME / kg metabolic weight) was sufficient to maintain SBW. Winter shearing increased dry matter intake. Different protein feeding levels had no effect on SBW indicating that all feeding levels supplied sufficient protein to the ewes. Winter shearing increased SBW although protein intake was comparable to the high protein feeding level indicating that SBW can be increased by an increasing supply of both energy and protein, or that the protein supply of shorn ewes might have been underestimated or that shearing altered the nutrient partitioning between mother and foetus. BCSC and wool growth were not affected by treatments, but shorn ewes lost more body weight during gestation. This was attributed to a higher deposition in SBW and higher energy requirements. It was concluded that a stable feed intake during pregnancy can be achieved by feeding a concentrate / roughage diet with increasing concentrate allowance, the Dutch protein requirements could be reduced and winter shearing was effective in improving lamb birth weight.

Key words : protein / requirements / utilisation / feed intake / gestation

Introduction

The Dutch DVE/OEB protein evaluation system for ruminants (Tamminga et al., 1994) calculates the amount of protein absorbed from the small intestine (DVE). For sheep DVE requirements were formulated from literature data (Everts, 1992) mainly derived with sheep breeds carrying small litters, but it is unclear whether or not these data apply to prolific ewes. Therefore, feeding trials with prolific ewes were considered necessary to check these requirements. Prolific ewes were introduced into sheep farming to improve the financial outcome of lamb meat production by increasing litter size. Large litter sizes (>2) often coincide with low lamb birth weights and with a reduction in feed intake during late gestation (Orr and Treacher, 1984 ; Everts, 1990). Low lamb birth weights are related to an increased lamb mortality before weaning (Dalton et al., 1980 ; McCutcheon et al., 1981 ;

Hinch et al., 1985) and impinges on postnatal growth and productivity (Bell, 1992). Improving birth weight of multiple birth lambs has potential for increasing lamb survival (Kleemann et al.,1990) and thus to improve the financial outcome of sheep farming.

Lamb birth weight can be manipulated by nutrition during pregnancy. A positive linear relationship between both energy- and protein intake and lamb birth weight exists (Robinson and Forbes, 1967). Extra protein- and energy intake during late pregnancy may result in a 34% increase of lamb birth weight (Stephenson and Bird, 1992). Studies with prolific Finn-cross ewes during late pregnancy showed that energy intake did not affect lamb birth weight, whereas an increased protein intake resulted in higher lamb birth weights (Everts, 1990). Other studies also showed that lamb birth weights can be increased by additional protein feeding in late pregnancy (Christenson and Prior,1976 ; Earl and Male, 1988 ; Kleemann et al., 1988 ; Lynch et al. 1990).

Lamb birth weights can also be increased by (winter) shearing during late pregnancy (Austin and Young, 1977 ; Maund, 1980 ; Thompson et al., 1982 ; Symonds et al., 1986 and 1992 ; Husain et al.,1997 ; Kenyon et al., 1999). The effect of winter shearing is not only due to an increased feed intake, because higher birth weights in shorn ewes were also observed when feed intake was kept constant (Rutter et al., 1972 ; Thompson et al., 1982 ; Symonds et al., 1986). The reason is more likely to be found in cold stress (Thompson et al., 1982) which can alter partition of some nutrients between mother and foetus in favour of the foetus. Cold exposure also improves the efficiency of utilisation of nutrients (Kennedy et al, 1986).

However, a direct comparison between the effects of protein feeding strategy and winter shearing during late gestation on reproduction performance of prolific ewes has not yet been made. Furthermore, it remains to be investigated how protein feeding strategy affects reproduction performances when prolific ewes are fed according to DVE requirements. Therefore, the present experiments were devised to investigate the effect of different DVE feeding levels and of (winter) shearing at approximately 6 weeks prior to parturition on reproductive performance of prolific ewes. The experiments also aimed to validate DVE requirements for prolific ewes during late gestation.

Materials and methods

Experiments and treatments

Two sequentially performed experiments were connected by a control treatment (C) to study the effects of protein supply and shearing during late gestation on the reproductive performance of prolific ewes. Each experiment was replicated over two years. Treatment C was kept constant over experiments during 4 years and involved ewes with an intact fleece

with protein and energy allowances according to requirements. For ewes in the last two months of gestation and carrying a triplet these requirements were 4.5 g DVE per kg metabolic live weight ($W^{0.75}$) (Everts, 1992) and 0.55 MJ ME / kg $W^{0.75}$ (CVB, 1992). In each experiment two treatments were compared to treatment C. During the first experiment (1992/1993) the effect of protein supply in ewes with an intact fleece was tested. Three DVE levels were fed: 0.8 (treatment PL), 1.0 (treatment C) and 1.2 (treatment PH) of requirements and energy supply was according to requirements. In the second experiment (1994/1995) the effect of shearing was investigated. This involved treatment C and two groups of shorn ewes. One group of shorn ewes was fed according to energy and protein requirements (treatment SC) and the other group of shorn ewes was offered a concentrate allowance comparable to that of the other two treatments but grass hay was fed *ad libitum* (treatment SAL).

Animals

Every year, eighty multiparous ewes of a prolific crossbred (Ile the France ♂ x Finnish Landrace ♀) were synchronised in oestrus and were mated with Texel rams. The day of mating was counted as day 0 of gestation. The animals were kept on pasture until the 85th day of gestation when they were group housed indoors. On the 95th day of gestation litter size was estimated from x-ray photographs. At day 102 of gestation sixty ewes were selected, shorn (if required by treatment) and individually penned. The shorn fleeces were weighed individually. The experimental period lasted from the 105th day of gestation and lasted until lambing.

Blocks of 3 comparable ewes were formed taking into account the estimated litter size, live weight, age and heritage. Treatments were allotted within blocks. The experimental design and number of ewes on each treatment are shown in Table 5.1.

The ewes were weighed weekly and body condition was scored (Jefferies, 1961) fortnightly. After lambing, ewes and lambs (including still born but fresh lambs) were weighed individually. The data from macerated and mummified lambs were excluded from the calculations.

In the first experiment the length of the wool was measured at the 105th and 140th day of gestation as the length of stretched wool. Wool length was measured at 3 locations on the back of the ewes. The front location was between the shoulder blades, the rear location was in the region of the pelvis and the middle location was in between the front rear location. Wool growth was calculated per location as the difference in length of the wool at the 105th and 140th day of gestation. Average wool growth was calculated as the mean of the three measurements.

Table 5.1
Experimental design and number of ewes per treatment

Treatment[1)]	C	PL	PH	SC	SAL
Experiment 1					
1992	20	20	20	-	-
1993	20	20	20	-	-
Experiment 2					
1994	20	-	-	20	20
1995	20	-	-	20	20
Replicates	80	40	40	40	40

[1)]Treatment C, PL and PH = respectively 1.0, 0.8 and 1.2 times protein requirements (CVB, 1992), SC = shorn and fed according to treatment C and SAL = shorn and *ad libitum* grass hay

Diets and feeding

The ewes were fed a grass hay / concentrate ration with a maximum of 750 grams of concentrates per day to prevent possible rumen acidosis. In 1992 the ewes were offered 1000 g grass hay (mainly *Lolium perenne*) daily. The calculated DVE content of the hays used in 1993, 1994 and 1995 was approximately 10% lower than the DVE content of the hay used in 1992. Therefore, in 1993, 1994 and 1995 hay allowance was increased to 1100 g grass hay daily, except for treatment SAL where hay was offered at approximately 15% refusals. The grass hay was supplemented with concentrates according to energy requirements based on the estimated litter size and the individual live weight of the ewes. The daily concentrate allowance was adapted weekly to actual ewe live weight (for the shorn ewes the weight of the fleece was added to the actual live weight). The protein content of the concentrates fed was according to treatments protein levels, which were established by feeding a mixture of two isocaloric concentrates, one with a low protein content and one with a high protein content. Each animal had individual access to fresh water. Grass hay and concentrates were fed twice daily (at 7.00 h and 15.00 h) and were offered separately but at the same time. Hay refusals were collected twice weekly and concentrate refusals daily.

Chemical analyses of samples

The samples were analysed following official Dutch protocols (Nederlands Normalisatie Instituut, 1992) (NEN), most of which are comparable with those of the Association of Official Analytical Chemists (AOAC, 1984). The analyses performed were dry matter

(DM, NEN 3332), ash (NEN 3329), nitrogen (N, NEN 3145) and crude fat (Publikatieblad EEG, 1984).

Statistical analysis

The statistical analysis was performed with the statistical package GENSTAT 5 (Payne et al., 1993). The results were analysed by multiple regression analysis. The model used was :

$$y = \mu + year_i + year_i.block_j + littersize_k + treatment_l + e_{ijkl}$$

where y = response variable
μ = general mean
$year_i$ = year effect (i = 1...4)
$block_j$ = block effect (j = 1...20)
$littersize_k$ = litter size (k=1...7)
$treatment_l$ = treatment effect (l = 1...5)
e_{ijkl} = error component assumed to be normally distributed with mean 0 and constant variance

This model resulted in three different standard error of differences (SED) for comparison of treatments : one SED for comparing the control treatment to the other treatments (this was the most precise comparison), one SED for comparing treatments within experiments and one SED for comparing treatments between experiments (this was the least precise comparison with a loss of approximately 0.30 in precision of the comparison with the control treatment). Before using this model, the results were first tested within experiments to check the comparability of the experimental variance between years. Then the validity of the model was checked by comparing the results on treatment effects to the results on treatments effects derived from models fit for the separate experiments.

Results

Chemical composition of the feeds used

The average chemical composition, in vitro digestibility of the organic matter (dOM), calculated metabolizable energy (ME) and calculated protein value (DVE) of hay and concentrates are represented in Table 5.2.

Table 5.2
Chemical composition (g/kg DM) of the feeds used

	H[1]				CLP[1]				CHP[1]			
	1992	1993	1994	1995	1992	1993	1994	1995	1992	1993	1994	1995
Dry matter (g/kg product)	888	860	894	865	878	888	862	863	871	881	881	876
Ash	104	101	107	100	53	56	67	68	58	66	77	73
Crude protein		184	182	192	141	73	70	102	118	301	366	355
Crude fat	35	28	21	23	42	30	58	65	39	48	58	60
Crude fibre	259	285	296	274	54	162	82	88	47	67	58	62
Sugar	---	---	---	---	112	70	66	90	75	69	91	103
Starch	---	---	---	---	309	312	344	345	261	137	143	147
Neutral detergent fibre	---	562	602	526	143	259	166	156	136	168	124	125
dOM[2]	0.73	0.71	0.71	0.74	0.87	0.88	0.83	0.84	0.85	0.85	0.84	0.85
ME (MJ/kg DM)	10.0	9.7	9.8	10.0	13.1	12.8	13.4	13.4	13.3	13.4	13.4	13.5
DVE	98	91	91	87	83	82	75	80	185	186	193	194

[1] H=hay, CLP=concentrate low protein and CHP=concentrate high protein
[2] digestibility of organic matter (Tilley and Terry, 1963)

Feed intake

Treatments C, PL and PH supplied different protein levels at comparable dry matter (DM) and energy levels. DVE intake of the ewes on treatment C was 93% of the aimed 4.5 g DVE / kg $W^{0.75}$, whereas DVE intake of ewes on treatment PL and PH was respectively 86% and 110% of the DVE intake on treatment C (Table 5.3). By experimental design, the ewes on treatment SC should have had an energy- and protein intake similar to that of the ewes on treatment C. Instead they showed significantly larger DM-, protein- and energy intakes than the ewes on treatment C. This was due to lower feed refusals than on treatments C, PL and PH.

Table 5.3
Comparison of feed intake between treatments in the experimental period (daily intake per kg metabolic weight, $W^{0.75}$). Averages with different indices differ significantly

	Experiment 1			Experiment 2	
Treatment[1)]	C	PL	PH	SC	SAL
DM (g)	43.0[a]	42.2[a]	42.1[a]	47.0[b]	52.2[c]
DVE (g)	4.2[a]	3.6[b]	4.6[c]	4.6[c]	5.0[d]
ME (MJ)	0.48[a]	0.47[a]	0.47[a]	0.52[b]	0.57[c]

[1)] Treatment C, PL and PH = respectively 1.0, 0.8 and 1.2 times protein requirements (CVB, 1992), SC = shorn and fed according to treatment C and SAL = shorn and *ad libitum* grass hay

Daily energy allowance was according to the ME requirement of triplet carrying ewes (0.55 MJ ME / kg $W^{0.75}$). The unshorn ewes were unable to ingest the amount of feed (especially grass hay) needed to meet the aimed ME intake, but the shorn ewes ingested larger quantities of grass hay. Nevertheless, only the shorn ewes with *ad libitum* grass hay were able to meet the aimed ME intake for unshorn ewes, but winter shearing increases ME demand for thermo regulation.

Reproductive performance

In total 234 of the 240 ewes gave birth to 792 lambs. Average litter size per lambing ewe was 3.38. Average lamb mortality at birth was 15.9% and total lamb mortality to 7 days after lambing was 17.7%. Average birth weight of lambs that survived the first 7 days after birth was 3.32 kg, while average birth weight of lambs that died at birth was 2.76 kg. Average birth weight of lambs that died during the first 7 days after birth was 2.66 kg. No significant differences in litter size were observed between treatments (Table 5.4). The

model used to test treatment effects on litter size was comparable to the model described in the materials and methods section except that litter size itself was not included.

Table 5.4
Average performance per treatment corrected for litter size (except litter size itself). $W^{0.75}$ [1] expressed as the average over the experimental period, BWC[1] as the difference between body weight at mating and directly after parturition, BCSC[1] as the difference between the condition score at day 108 and day 136 of gestation and wool growth as the average over the period between 105 and 140 days of gestation. Averages with different indices differ significantly

Treatment[2]	C	PL	PH	SC	SAL
$W^{0.75}$ [1] of the ewe (kg)	28.7 [a]	28.8 [a]	28.8 [a]	27.8 [b]	28.1 [ab]
Litter size	3.4 [a]	3.3 [a]	3.2 [a]	3.5 [a]	3.4 [a]
SBW[1] (kg)	10.68 [a]	10.84 [ab]	10.45 [a]	11.64 [b]	11.25 [ab]
Body weights of the ewes					
At mating (kg)	75.6 [a]	76.5 [a]	75.6 [a]	73.4 [a]	74.4 [a]
After parturition (kg)	75.7 [a]	78.4 [a]	78.2 [a]	70.3 [b]	72.2 [b]
BWC[1] (kg)	0.4 [ab]	2.2 [a]	2.6 [a]	-2.9 [c]	-2.0 [bc]
Net loss at parturition (kg)	16.0 [a]	14.9 [a]	14.2 [a]	18.4 [b]	16.8 [ab]
BCSC[1]	-0.16 [a]	-0.14 [a]	-0.12 [a]	-0.15 [a]	-0.13 [a]
Wool growth (mm)	12.0 [a]	14.9 [a]	14.9 [a]	-	-

[1] $W^{0.75}$ = metabolic weight , BWC = body weight change, BCSC = body condition score change, SBW = sum of lamb birth weights within litters

[2] Treatment C, PL and PH = respectively 1.0, 0.8 and 1.2 times protein requirements (CVB, 1992), SC = shorn and fed according to treatment C and SAL = shorn and *ad libitum* grass hay

Because lamb birth weight is confounded with litter size, the results for lamb birth weights were analysed as the sum of birth weights (SBW) of lambs born within one litter (Table 5.4). The average SBW was not affected by treatments PL and PH, but shearing increased SBW significantly. The average SBW of litters born to shorn ewes was not affected by the level of grass hay supplied.

Body weight and condition of the ewes

Between treatments C, PL, PH and SAL no significant differences in metabolic live weight were observed (Table 5.4). Nevertheless, shorn ewes tended to have lower live weights

(with statistical significance for treatment SC) than unshorn ewes, but shorn ewes had also lower live weights (not significant) at mating. Mean body weight of the ewes at mating was 75.1 kg and did not differ between treatments (Table 5.4). Directly after parturition the mean body weight was still approximately 75 kg, but the average body weight after parturition of shorn ewes (71.2 kg) was significantly lower than that of unshorn ewes (77.4 kg). This was due to the fact that winter shearing resulted in a negative body weight change (BWC) of the ewes during gestation. BWC of shorn ewes that were fed hay *ad libitum* tended to be lower than BWC of unshorn ewes on treatment C. BWC was calculated as the difference between body weight at mating and body weight directly after parturition under the assumption that body weight gain from mating until the start of the experimental period was comparable between treatments. When expressed as net loss at parturition (calculated as body weight before parturition minus body weight after parturition) these differences due to shearing resulted in a higher net loss in shorn ewes (statistically significant for SC). This larger net loss in shorn ewes was not reflected in the change in body condition score of the ewes.

Discussion

Feed intake

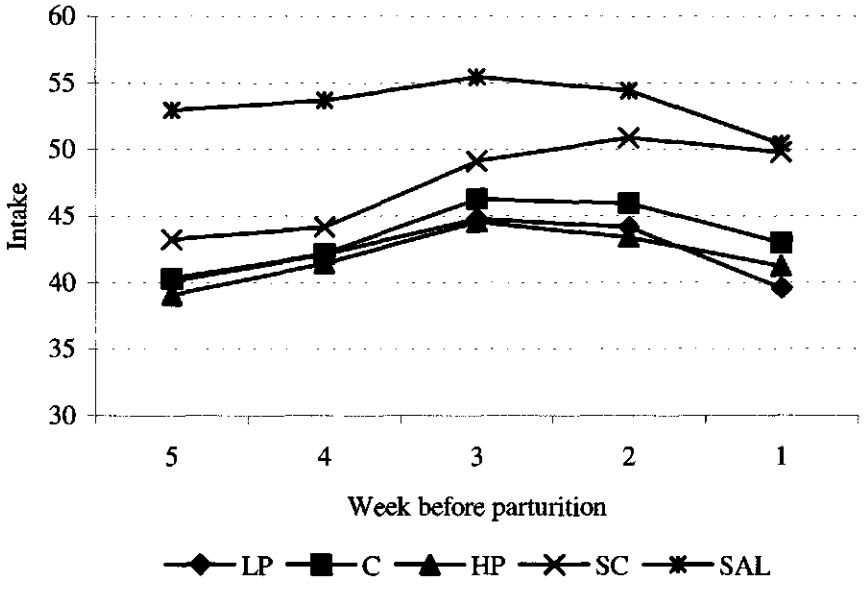
The average feed intake during the experimental period was expected to decrease with advancing pregnancy, since ewes carrying large (>2) litter sizes were used. Feed intake is often reduced during late gestation for ewes carrying large litter sizes (Orr and Treacher, 1984 ; Everts, 1990). Nevertheless, average daily DM intake remained stable until the 143th day of gestation (Figure 5.1), but the intake of grass hay decreased with increasing allowance (and intake) of concentrates during the experimental period. This observation is in agreement with Barry and Manley (1985) who concluded that intake in late pregnancy is either stable or falls with advancing pregnancy due to a reduced rumen volume as a result of the expanding uterus.

ME intakes exceeded 0.45 MJ / kg $W^{0.75}$ on all treatments which is sufficient to maintain lamb birth weights in Finn-cross ewes (Everts, 1990). Although feed intake during the last six weeks of pregnancy was lowest for the unshorn ewes, they were able to increase or at least maintain maternal body weight during pregnancy. Both observations indicate that the nutritional requirements of the ewes were met. This is in contrast to the findings of Robinson (1990) who concluded for prolific ewes that the capacity of the digestive tract was insufficient to meet the nutritional requirements, even if the diet was of the highest quality.

The feed intake of roughage diets is principally ruled by rumen outflow rate and rumen degradation rate (Waldo, 1985), and both rumen outflow rate (Kennedy et al., 1986) and

rumen degradation rate (Šebek and Everts, 1999) were found to increase with winter shearing. In the present experiments feed intake was indeed increased by winter shearing and increased even further when grass hay was offered *ad libitum*.

Figure 5.1
Daily dry matter intake (g per kg metabolic weight) during the last 5 weeks of gestation. Treatment LP=low protein, C=control, HP=high protein, SC=shearing and fed as treatment C, SAL=shearing and fed *ad libitum* grass hay



Reproductive performance and protein intake level

Reproductive performance was measured as the sum of birth weights (SBW) of the lambs within each litter. SBW is related to litter size (Everts, 1990) and thus litter size was included in the statistical model to test the effects of treatment on SBW. SBW can be affected by nutrition during late gestation (McNeill et al., 1992 ; Stephenson and Bird, 1992 ; Lynch et al., 1990 ; Everts, 1990 and Kleemann et al., 1988), but SBW can also be affected by nutrition during early (Orleans-Pobee and Beatson, 1989) and mid pregnancy (Mellor, 1983). Nutrition during early pregnancy affects the number of placentomes that are formed (fixed at approximately 30 days of gestation) and nutrition during mid pregnancy affects the total weight of the placentomes until approximately 90 days into gestation (Mellor, 1983). In the present experiments all animals received the same (nutritional)

treatment until the 105th day of gestation. Therefore, the influence of placental development (combined result of number and weight of placentomes) on SBW was assumed not to interfere with the influence of nutritional treatment during late gestation.

In contrast with findings described in literature (Christenson and Prior,1976 ; Earl and Male, 1988 ; Kleemann et al., 1988 ; Lynch et al. 1990; Everts, 1990), SBW was not affected by the level of protein ingestion. This observation indicates that all treatments supplied sufficient protein to the ewes since low protein intakes result in less protein accretion in foetuses, whereas high protein intakes do not result in a further increase of protein accretion in foetuses (McNeill et al.,1992). However, the findings of McNeill et al. (1992) do not seem to apply to shorn ewes, because winter shearing increased SBW even though DVE intake of ewes on treatment SC was comparable to those on treatment PH. The observed increase in SBW can not be due to the higher energy intake on treatment SC since supplementation of energy only does not affect SBW (Stephenson and Bird, 1992 ; Everts, 1990). However, SBW can be increased by intake of extra protein and energy (Stephenson and Bird, 1992). The observed increase in SBW at comparable protein intake levels could also indicate that DVE supply was underestimated in shorn ewes. This possible underestimation of DVE may be due to the fact that DVE calculation does not take into account that cold exposure (winter shearing) increases the efficiency of microbial growth in the rumen (Kennedy et al., 1986 ; Kennedy and Milligan, 1978) and outflow of protein from the rumen (Kennedy et al., 1986). Another explanation for the observed increase in SBW due to winter shearing is an altered partitioning of nutrients between mother and foetus (Thompson et al.,1982).

Body weight, condition of the ewes and wool growth

The effects of treatments could also be seen in ewe body weight change (BWC), the change in body condition score (BCSC) or in wool growth. None of these parameters were affected by treatments, except for BWC in shorn ewes.

The negative BWC in shorn ewes resulted from a higher energy deposition in SBW of the lambs. In addition shorn ewes may have had higher energy requirements for maintaining body temperature. The negative BWC of shorn ewes may also be due to a redistribution of nutrients. This redistribution of nutrients may result from mobilisation of protein from the ewe which is subsequently deposited in the lambs. This would be in agreement with Frutos et al. (1998), who concluded that BWC was a better predictor of protein mobilisation, while BCSC was a better predictor for fat metabolism.

The lack of an effect of protein ingestion level on wool growth is in agreement with the SBW results. Wool growth is more sensitive to a reduced supply of protein than foetal growth, although degradable protein (i.e. microbial protein) does not affect wool growth

(Masters et al., 1996). In our experiments protein intake levels were established by concentrates which were degradable (Šebek and Everts, 1999). The apparent lack of effect on wool growth may be due to a comparable intake of undegradable protein.

Conclusions

Dry matter intake from a concentrate / roughage diet was stable during late pregnancy when concentrate allowance was increased with advancing pregnancy.

Different DVE intake levels did not affect reproductive performance nor parameters of ewe performance. Therefore, protein intakes at the level of the average intake of the low protein treatment (3.6 g DVE / kg $M^{0.75}$) seem sufficient to meet requirements of ewes during late gestation with a metabolic live weight of approximately 29 kg and carrying triplets. Current DVE requirements (4.5 g DVE / kg $M^{0.75}$) could therefore be reduced.

Winter shearing was effective in improving lamb birth weight, whether or not roughage was offered *ad libitum*. This might be due to the combined effect of increased feed intake, increased efficiency of (protein) degradation and an alteration in nutrient partitioning between mother and foetus.

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Chapter 6

Protein requirement and protein utilisation of crossbred ewes during early lactation

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Abstract

Two feeding trials were used to investigate the effects of different protein feeding levels and of different energy allowance levels during the first six weeks of lactation on milk production performance, on mobilisation of body tissues and on the efficiency of ingested protein in milk protein production. The protein feeding levels supplied 90%, 100% and 110% of expected requirements and the energy feeding levels supplied 80% and 100% of expected requirements. Each diet consisted of grass hay and concentrates. Milk production performance was not affected by protein feeding levels when energy allowance was approximately 80% of the expected requirements, but milk production performance increased with increasing protein intake at an energy allowance of approximately 100% of the expected requirements. This was considered to be a result of plateau level production at constant energy allowance. The calculated energy deficit in early lactation diminished as energy allowance increased, whereas body weight change and body tissue mobilisation decreased. From fat and protein mobilisation estimations it was concluded that energy supply was limiting milk production in the experiments at 80% energy allowance, whereas protein supply was limiting milk production at 100% energy allowance. The efficiency of milk protein production was not affected by energy allowance level. This could be due to differences in mobilised energy. It was concluded that the lowest protein ingestion level and the 80% energy allowance were sufficient to maintain milk production performance.

Keywords: protein / requirements / utilisation / lactation

Introduction

A new protein evaluation system for ruminants, the DVE/OEB system, was introduced in the Netherlands (Tamminga et al., 1994) and validated for dairy cows. In order to adapt the system for sheep it was necessary to investigate the requirements for true protein absorbed from the small intestine (DVE) of sheep. For lactating ewes, protein requirements depend on the amount of protein needed for maintenance and for milk protein production. Literature is relatively consistent about the amount of protein required for maintenance and the value from the AFRC (1995) can be adopted. The amount of protein needed for milk production depends on the efficiency by which DVE is used for milk protein production and on the amount of milk protein produced. The average efficiency of milk protein production in ewes is 0.68 (ARFC, 1995), but shows a large variation (Everts, 1992). Experiments showed that ewes with high protein intakes had average efficiencies of milk protein production of 0.67 (Papas, 1977) and 0.63 (Ngongoni et al.,1989), whereas ewes with low protein intakes had an average efficiency of milk protein production of 0.85 (Robinson and Forbes, 1970). The relationship between protein ingestion and milk protein production is not linear and reaches a plateau level (Gonzales et al., 1984). In order to be able to determine the efficiency of milk protein production by regression analysis,

information on milk protein production at different levels of protein ingestion is required alongside requirement estimates for protein maintenance and mobilisation (or deposition).

Another factor influencing (efficiency of) milk protein production, is the level of energy supply to the ewe. During early lactation energy intake seldom enables sufficient milk production for desirable growth of twin lambs (Robinson, 1980). Diets normally offered to sheep in a system with one lambing season per year cover approximately 80% of the energy requirements during the first six weeks of lactation. The remaining 20% must come from mobilisation of body reserves, which results in live weight losses during early lactation. In such a situation, protein supplements enables the ewe to augment the energy deficit by the mobilisation and subsequent safe utilisation of even more body fat and thus milk production will increase with increasing protein intake (Robinson, 1980 ; Cowan et al., 1981). This increase in milk (protein) production reaches a plateau level, the plane of which is positively related to energy intake (Gonzales et al., 1984). It is therefore necessary to investigate (the efficiency of) milk protein production at different levels of energy ingestion.

The objective of the present study was to investigate the effects of DVE feeding levels on milk production, on the efficiency of milk protein production, on body weight change and on mobilisation of body tissues in crossbred ewes during early lactation. The effect of energy intake was investigated by comparing the results of two experiments. These experiments were identical except for the level of energy supply.

Materials and methods

Experiments

Two experiments were performed with prolific ewes during the first six weeks of lactation to study the effects of three DVE levels on milk production, on the efficiency of milk protein production, on ewe body weight change and on the ewes body tissue mobilisation. Each experiment was replicated over two years. The experiments were conducted in consecutive years.

Experiment 1

Treatments consisted of three DVE allowance levels at a common practice energy allowance level of approximately 0.8 times requirement (CVB, 1992). The DVE allowance levels supplied 0.9 (treatment PL), 1.0 (treatment C) and 1.1 (treatment PH) times requirement (CVB, 1992). The DVE requirements were calculated for ewes of 70 kg live weight, producing 140, 145, 135, 120, 115 en 105 grams of milk protein per day in the first to the sixth week of lactation respectively. The energy requirement of these ewes was

calculated to be 29.8 MJ ME per day in the first 3 weeks of lactation and 26.4, 25.6 and 23.2 MJ ME per day in respectively week 4, 5 and 6 of lactation (AFRC, 1995).

Experiment 2
This experiment was identical to experiment 1, except that the energy allowance level was not 0.8 times requirements but according to the above mentioned energy requirements (CVB, 1992).

Animals

Every year 22 multiparous ewes (with 2 lambs) of a highly prolific crossbred (Ile the France ♂ x Finnish Landrace ♀) were used, except in the first experimental year when 21 ewes were used. Each ewe suckled two lambs. In the first experiment 43 ewes and 86 lambs were used and in the second experiment a total of 44 ewes and 88 lambs. Each year the ewes were selected from a group of 60 animals that were synchronised in oestrus and mated with Texel rams. The ewes for the experiments were selected after lambing based on lambing date, vitality of the ewe and the lambs, suckling behaviour of the lambs, live weight of the lambs, sex of the lambs and feed intake of the ewes. The ewes were housed indoors in individual pens. The experiments started the first Monday after parturition, hence the ewes were 4 to 7 days in lactation at the start of the experimental period.

Blocks of 3 comparable ewes were formed taking into account the sex of the lambs and live weight of lambs and ewe. The three treatments were allotted within blocks. During the last 3 experimental years one block consisted of 4 ewes and this extra ewe was allotted randomly to a treatment. The experimental design and the number of ewes on each treatment are shown in Table 6.1.

Table 6.1
Number of ewes per treatment

Treatment[1)]	PL	C	PH
Experiment 1: energy allowance of 0.8 requirements (CVB, 1992)			
1992	7	7	7
1993	7	8	7
Experiment 2 : energy allowance of 1.0 requirements (CVB, 1992)			
1994	8	7	7
1995	7	7	8

[1)]Treatment C, PL and PH = DVE allowance of respectively 1.0, 0.9 and 1.1 times requirements (CVB, 1992)

Diets and feeding

In experiments 1 and 2 the ewes were offered a daily allowance of 1300 and 1400 grams of grass hay (mainly *Lolium perenne*), respectively. The roughage was supplemented with concentrates according to actual energy requirements. The daily concentrate allowance was adapted each week to the stage of lactation: for experiments 1 and 2 in the first three weeks of lactation respectively 1100 and 1500 grams concentrates daily, thereafter every week 100 grams less per day. The protein content of the concentrates was in accordance with treatment protein levels, which were established by feeding a mixture of two isocaloric concentrates, one with a low protein content (CLP), the other with a high protein content (CHP). The animals had individual (free) access to fresh water. Roughage and concentrates were fed twice daily (at 7.00 h and 15.00 h) and were offered separately but at the same time. Hay refusals were collected twice weekly and concentrate refusals daily. From week 3 of lactation onwards the lambs were offered additional grass hay and concentrates in a pen next to the ewe. These pens were separated by a fence that restricted access of the ewe but allowed passage of the lambs. Every week the daily hay intake of the lambs was measured during a 24-hour period in which ewe and lambs were separated by a closed fence. The daily hay ingestion of the ewes was corrected for the amount of hay consumed by the lambs as observed on the day of milk yield measurements. The chemical composition and *in vitro* digestibility of the organic matter (dOM) were analysed and the metabolizable energy (ME) content and protein value (DVE) of hay and concentrates was calculated (Table 6.2).

Milk yield and milk composition

Milk yield was measured each week over a period of 24 hours, except for the second year in experiment 1 when milk yield was measured over a 16-hour period. During the period of milk yield measurement the lambs were separated from the ewe, but were allowed to suckle every 4 hours. Just before and immediately after sucking the lambs were weighed and milk production was assumed to be the difference between these two bodyweights. After each period of milk production measurement of 24 hours the lambs remained separated from the ewe for a further 4 hours. Then the ewe received an intravenous injection in the jugular vein of 5 i.u. of oxytocin immediately followed by hand milking. The amount of hand-milked sample differed per ewe and was approximately 1/6 of the ewe's milk production over the previous 24 hours. The hand-milked sample was sub-sampled to determine milk composition (Šebek and Everts, 1993). With this protocol it was assumed that the composition of the handmilked sample reflected the composition of the milk during suckling.

Table 6.2
Chemical composition (g/kg DM) of the feeds used

	Grass hay				Concentrate CLP[1)]				Concentrate CHP[1)]			
	1992	1993	1994	1995	1992	1993	1994	1995	1992	1993	1994	1995
Dry matter (g/kg product)	888	860	894	865	878	888	862	863	871	881	881	876
Ash	104	101	107	100	53	56	67	68	58	66	77	73
Crude protein	184	182	192	141	73	70	102	118	301	366	355	352
Crude fat	35	28	21	23	42	30	58	65	39	48	58	60
Sugar	---	---	---	---	112	70	66	90	75	69	91	103
Starch	---	---	---	---	309	312	344	345	261	137	143	147
Neutral detergent fibre	---	562	602	526	143	259	166	156	136	168	124	125
dOM[2)]	0.73	0.71	0.71	0.74	0.87	0.88	0.83	0.84	0.85	0.85	0.84	0.85
ME (MJ/kg DM)	10.0	9.7	9.8	10.0	13.1	12.8	13.4	13.4	13.3	13.4	13.4	13.5
DVE	98	91	91	87	83	82	75	80	185	186	193	194

[1)] CLP = concentrate with low protein content and CHP = with high protein content
[2)] digestibility of organic matter (Tilley and Terry, 1963)

Observations

The ewes were weighed weekly and their body condition (Jefferies, 1961) was scored fortnightly. Milk production was measured weekly as described previously. The feed intake of the ewes was calculated by subtracting grass hay and concentrate leftovers from their feed allowance.

Calculations

The energy deficit of the ewe was calculated from the difference between milk production based on the available ME and actual ME used for milk production. The milk production based on available ME was calculated as ME intake minus ME necessary for maintenance and the efficiency by which this energy is used for milk production was calculated according to AFRC (1995). The maintenance requirement for ME (including activity allowance and fleece growth) was also calculated according to AFRC (1995). The gross energy (GE) output with milk was calculated with the regression equation provided by Šebek and Everts (1993). Finally, the energy mobilisation was calculated assuming an efficiency of 0.85 for the utilisation of mobilised energy for milk production (Van Es, 1978).

Body protein and fat mobilisation was calculated on the following theoretical assumptions:
a) 1 gram of mobilised protein is equivalent to 4 grams of mobilised live weight,
b) 1 gram of mobilised fat is equivalent to 1.1 grams of mobilised live weight,
c) the energy content of 1 gram protein is 23.8 kJ and
d) the energy content of 1 gram fat is 39.5 kJ.
These assumptions, the measured body weight change (BWC) and the calculated amount of mobilised energy resulted in equations (1) and (2) with two unknown parameters (fat and protein). Substitution of equation (1) into equation (2) provides an estimation of the amounts of fat and protein mobilised from the body.

$$\text{BWC (g / day)} = 4.0 \text{ protein (g)} + 1.1 \text{ fat (g)} \quad (1)$$

$$\text{Mobilised energy (kJ / day)} = 23.8 \text{ protein (kJ)} + 39.5 \text{ fat (kJ)} \quad (2)$$

The efficiency of the utilisation of DVE available for milk production (i.e. above maintenance) was estimated by regression analysis. Equation (3) is the model used to fit the relationship between DVE intake above maintenance requirement (DVEm+), milk protein production and mobilised protein:

$$\text{DVEm+} = b_1 * \text{milk protein} + b_2 * \text{mobilised protein} + e \text{ (g / kg } W^{0.75} \text{ per day)} \quad (3)$$

Where b_1 and b_2 are regression coefficients and e is the residual variance. The model omitted the constant (b_0) because the relationship between DVEm+ intake, milk protein production and mobilised protein is forced through the origin.

Chemical analyses of samples

Milk samples were analysed for fat, protein and lactose content by infrared spectrometry as described by Šebek and Everts (1993). The feed samples were analysed for DM, ash, N, fat, NDF, starch and sugar contents as described by Van Vuuren, et al. (1993). NDF was assayed without sodium sulphite, with alpha amylase and without residual ash. The in vitro digestibility of organic matter was analysed according to Tilley and Terry (1963).

Statistical analysis

The statistical analysis was performed with the statistical package GENSTAT 5 (Payne et al., 1993). The results were analysed by multiple regression analysis. The model used per experiment was:

$$y = \mu + year_i + block_j + year_i.block_j + treatment_k + e_{ijk}$$

where y = response variable
μ = general mean
$year_i$ = year effect (i = 1...2)
$block_j$ = block effect (j = 1...7)
$treatment_k$ = treatment effect (k = 1...3)
e_{ijk} = error component assumed to be normally distributed with mean 0 and constant variance

Results

Feed intake

Treatments C, PL and PH supplied different protein levels at comparable DM and energy allowance levels. During experiment 1, the average DVE intake of the ewes on treatment C was at the aimed level of 9.8 g DVE / kg $W^{0.75}$, whereas the ewes on treatment PL and PH ingested respectively 0.89 and 1.09 times the DVE intake on treatment C (Table 6.3). In experiment 2 these ratio's were (0.99) the aimed level of DVE intake for treatment C. Fortreatments PL and PH these were respectively 0.92 and 1.12 times the DVE intake on treatment C.

In experiment 1 the ewes ingested, as intended, on average 0.82 times the calculated 100% energy requirement for a 70 kg ewe. The increase in concentrate allowance in experiment 2, enabled the ewes to ingest almost all (0.99) of the calculated amount of 100% energy requirement for ewes of 70 kg live weight (Table 6.3).

Table 6.3
Feed intake during the experimental period; averages expressed as daily intake per kg metabolic weight. Averages with different indices differ significantly.

	Treatment[1)]		
	PL	C	PH
Experiment 1 : ME allowance 80% of requirements			
DM (g)	79.4	80.9	80.6
DVE (g)	8.6 [a]	9.80 [b]	10.68 [c]
ME (MJ)	0.92	0.94	0.94
Experiment 2 : ME allowance 100% of requirements			
DM (g)	94.7	94.0	97.0
DVE (g)	8.95 [a]	9.72 [b]	11.03 [c]
ME (MJ)	1.12	1.11	1.15

[1)]Treatment C, PL and PH = respectively 1.0, 0.9 and 1.1 times protein requirements (CVB, 1992)

Milk production performance, body weight and condition of the ewes

In the first experiment milk production was 7% higher than expected (Figure 6.1) for all treatments, but milk protein production was as expected (Figure 6.2). In experiment 1, treatments did not affect milk production performance (Table 6.4). In the second experiment milk production on treatment PL was as expected, but on treatments C and PH milk production was respectively 7% and 11% higher than expected. The results for milk protein production were comparable to the results for milk production. From week 4 of lactation onwards milk production and milk protein production on treatment PH were significantly higher than milk production on treatment PL. The increased milk production on treatment PH resulted in significantly higher production of milk fat, milk protein and GE in milk than on treatment PL.

Comparison between experiments 1 and 2 is invalid, due to year and animal differences. The higher energy allowance in experiment 2 did not result in a higher milk production.

Figure 6.1
Milk production per kg metabolic weight during early lactation

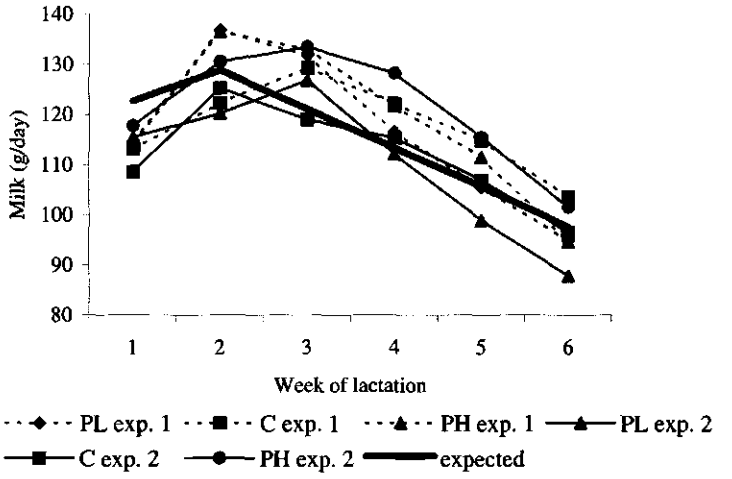


Figure 6.2
Milk protein production per kg metabolic weight during early lactation

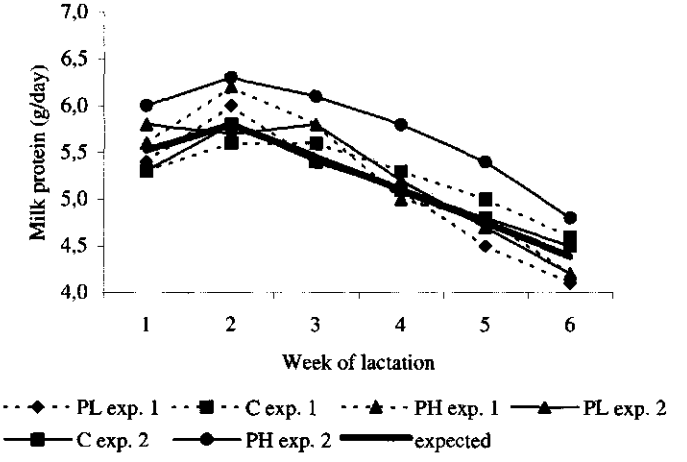


Table 6.4
Daily milk production performance (expressed per kg metabolic weight) during the experimental period. Averages with different indices differ significantly.

	Treatment[1)]		
	PL	C	PH
Experiment 1 : ME allowance 80% of requirements			
Milk (g)	119	121	121
Milk fat (g)	9.7	9.8	9.5
Milk protein (g)	5.3	5.4	5.4
Milk lactose (g)	5.9	5.9	5.9
Gross energy in milk (kJ)	618	622	610
Experiment 2 : ME allowance 100% of requirements			
Milk (g)	113	114	123
Milk fat (g)	9.3[a]	9.8[ab]	10.8[b]
Milk protein (g)	5.4[ab]	5.2[a]	5.8[b]
Milk lactose (g)	5.5	5.5	5.9
Gross energy in milk (kJ)	594[a]	611[ab]	674[b]

[1)]Treatment C, PL and PH = respectively 1.0, 0.9 and 1.1 times protein requirements (CVB, 1992)

Mobilisation of body tissues

In both experiments DVE ingestion levels did not affect BWC or BCSC (Table 6.5). The calculated energy mobilisation was not affected by treatments in experiment 1, but tended to increase from treatment PL to PH (P =0.06) in experiment 2.The extra energy allowance in experiment 2 resulted in a lower calculated energy mobilisation, in lower body weight losses and a smaller decrease in body condition. In experiment 1, treatments did not affect the mobilisation of body tissues, although the amount of mobilised protein seemed to decrease with increasing DVE ingestion level (Table 6.5). In experiment 2 treatments affected mobilisation of body tissues. The amount of mobilised protein decreased and became protein deposition with increasing amounts of ingested DVE. The amount of mobilised fat also increased with increasing amounts of ingested DVE.

Table 6.5
Observed body weight change (BWC), body condition score change (BCSC) and calculated mobilisation of body tissues and energy ; mobilisation calculated from BWC, calculated energy deficit and theoretical energy content of body protein and body fat. Averages with different indices differ significantly.

	Treatment[1)]		
	PL	C	PH
Experiment 1 : ME allowance 80% of requirements			
BWC (g/day)	-214	-190	-187
BCSC	-0.22	-0.22	-0.22
Mobilised energy (kJ/day)	7330	6879	7133
Mobilised protein (g/day)	6	-2	-4
Mobilised fat (g/day)	187	179	187
Experiment 2 : ME allowance 100% of requirements			
BWC (g/day)	-136	-119	-116
BCSC	-0.17	-0.17	-0.18
Mobilised energy (kJ/day)	3042	3893	4950
Mobilised protein (g/day)	15^{a}	3^{ab}	-6^{b}
Mobilised fat (g/day)	68^{a}	97^{ab}	129^{b}

[1)]Treatment C, PL and PH = respectively 1.0, 0.9 and 1.1 times protein requirements (CVB, 1992)

Efficiency of milk protein production

The regression equation between DVE intake above maintenance (DVEm+), milk protein production and protein mobilisation (expressed per kg $W^{0.75}$) was used separately for ewes with protein mobilisation and for ewes with protein deposition . The performed regression analyses accounted for a low percentage of variance in experiments 1 and 2 of 23 and 25% for ewes mobilising protein and of 0 and 14% for ewes depositing protein respectively. In the first experiment approximately half of the ewes were mobilising protein over the first six weeks of lactation, whereas in the second experiment approximately one third of the ewes were mobilising protein. These results show that the efficiency by which ingested protein was used for milk protein production was higher for ewes with protein mobilisation than for ewes with protein deposition. This difference was larger in experiment 1 (0.639 ± 0.04 vs. 0.587 ± 0.04) than in experiment 2 (0.645 ± 0.03 vs. 0.630 ± 0.04).

Discussion

Feed intake

During the first 6 weeks of lactation milk production (Figure 6.1) was one week ahead of feed intake (Figure 6.3). After the third week of lactation concentrate allowance was diminished gradually, which resulted in a decline in DM intake.
The present experiments showed that crossbred ewes increased DM ingestion due to an increased amount of concentrates in the diet, despite an expected limitation to DM intake in early lactation (Robinson, 1980). This resulted in a lower energy mobilisation in experiment 2 than in experiment 1 (Figure 6.4).

Milk production performance and protein intake level

The observed milk production curve (Figure 6.1) was characteristic for ewes (Gardner and Hogue, 1964 ; Rattray, 1992), indicating that the ewes were able to meet their demands by nutrition and mobilisation (Susin et al., 1995). In both experiments the average milk production during the first six weeks of lactation was about 6% higher than anticipated. However, in experiment 1 the ewes on all treatments produced about 6% more milk than expected, whereas in experiment 2 the ewes on treatments PL, C and PH produced 100%, 107% and 111% of the expected milk production. The increase in milk production in experiment 2 was positively related to the increase in DVE ingestion according to treatments, where the ewes on treatment PH produced significantly more milk than the ewes on treatment PL from the third week of lactation onwards. This difference was not significant to over-all milk production of the first six weeks of lactation, but resulted in a significant increase in the over-all production of milk fat, milk protein and GE in milk on treatment PH (Table 6.4). Often extra protein intake increases milk production (Penning et al., 1988 ; Sheehan and Hanrahan, 1989), but not always (Hatfield et al., 1995). At constant energy intake levels, extra protein intake will increase milk production, until a plateau level is reached (Robinson, 1980; Gonzales et al., 1984). This plateau level increases with energy intake level (Robinson, 1980; Gonzales et al., 1984). Thus, the different effect of protein ingestion levels on milk production in experiment 1 and 2 can be explained by an increased plateau level of milk production due to the higher ME intake in experiment 2. Apparently the ewes in experiment 1 were producing at their plateau level on all treatments, whereas the ewes in experiment 2 were not. However, the milk production between experiments was more or less comparable, whereas it was expected that the ewes in experiment 2 would produce more milk due to the higher amount of concentrates in the diet. Increased milk production due to extra concentrates in the diet is reported by Cowan et al.(1980) and Susin et al.(1995). This increase is not only due to a higher energy intake (Cowan et al., 1980), because it was also seen at comparable energy intakes (Susin et al., 1995). A possible explanation for the latter is an altered ruminal VFA production (Susin et al., 1995), whereas

Figure 6.3
Dry matter intake per kg metabolic weight during early lactation

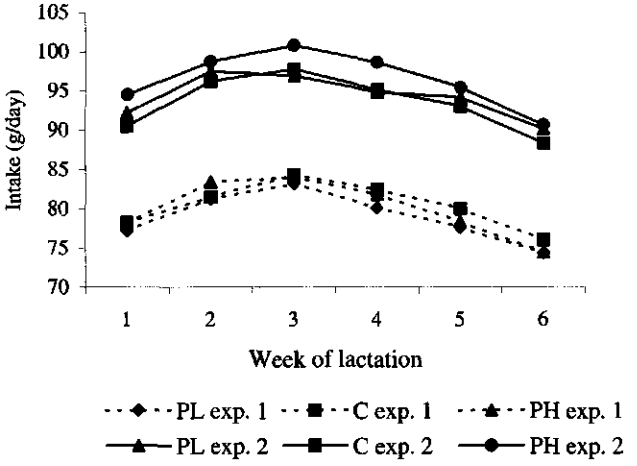
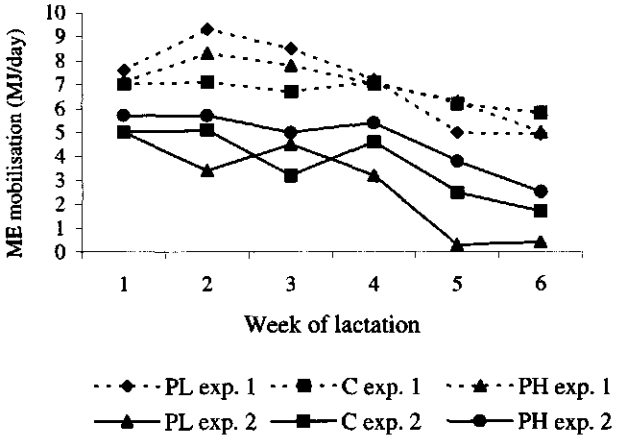


Figure 6.4
Mobilisation of metabolic energy (ME) during early lactation



it is difficult to speculate on a higher efficiency of digestion for diets with large amounts of concentrates at high intake levels (Sheehan and Hanrahan, 1989). It must be emphasised that the comparison of milk production level between the two experiments is invalid due to differences between experimental years and animals.

Body weight and condition of the ewes

BWC can be used as a predictor of protein mobilisation and BCSC can be used as a predictor for fat metabolism (Frutos et al., 1998). In the present study BCSC indicated that in both experiments body fat was mobilised and that the ewes in experiment 2 mobilised less fat than those in experiment 1. This is in agreement with the calculated fat mobilisation (Table 6.5). BWC was in agreement with the calculated protein mobilisation or deposition with regard to the comparison of treatments within experiments. However, BWC was not indicative for the differences in protein deposition between experiments. An increased protein intake without a change in ME intake often results in an increased milk production and an increased live weight loss in the ewe (Sheehan and Hanrahan, 1989 ; Hatfield et al., 1995). Both effects were not observed in experiment 1, which sustains the conclusion that the ewes in experiment 1 were producing at their plateau level. In experiment 2 increased protein intake resulted in a milk production increase, but BWC decreased instead of increasing.

Mobilisation of body tissues

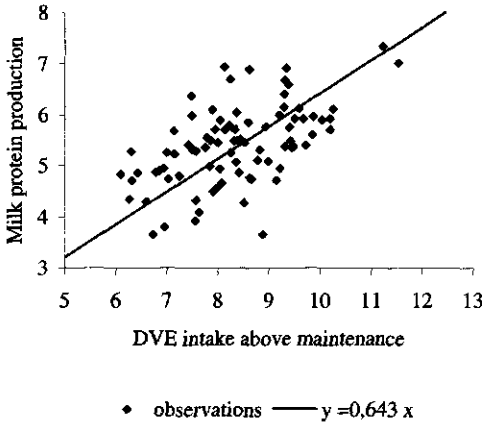
The treatments in experiment 1 did not affect calculations of amounts of deposited protein and mobilised fat, but in experiment 2 a statistically significant change was observed from protein mobilisation (treatment PL) into protein deposition and fat mobilisation increased as protein intake level increased. This resulted in a decrease in the ratio between mobilised protein and fat and thus in an increased energy content of the mobilised body tissue. This is in agreement with Cowan et al. (1980) who concluded that BWC in early lactation is not a reliable indicator of changes in body energy. The observed protein deposition during the period of negative energy balance was also reported in dairy cows (Tamminga et al., 1997) and growing lambs (Chowdhury et al., 1997). From the similarity in calculated fat mobilisation between treatments in experiment 1 (Table 6.5) it can be concluded that energy supply was limiting milk production and not protein supply, which is in agreement with milk production at a plateau level. Therefore, milk production was not affected by protein ingestion level in experiment 1. In experiment 2, the energy needed for the increasing milk production with increasing protein intake was supplied by an increasing fat mobilisation (Table 6.5). This observation indicated that in experiment 2 protein supply was limiting milk production and not energy supply, which is in agreement with the conclusion that these ewes were not producing milk at a plateau level. However, it must be emphasised that the theoretical approach of the calculation of body tissue mobilisation

relies on a good estimation of the calculated energy deficit and of the BWC. For dairy cows it is known that BWC should be corrected with a gut fill factor of 4 kg per kg of dry matter intake (Jarrige, 1989). However, it is unclear how this gut fill factor relates to sheep. In addition, dry matter intake (DMI) differences between the first and sixth week of lactation in the present experiments was about 100 – 200 g per day. Therefore, BWC was not corrected for DMI.

Efficiency of utilisation of DVE for milk protein production

The estimated efficiencies of the utilisation of DVE for milk protein production in relation to the energy ingestion level were in agreement with the results from other experiments (Robinson and Forbes, 1970; Papas, 1977; Ngongoni et al., 1989). The highest efficiencies were calculated for the ewes mobilising protein. In practice, it will be most profitable to feed lactating *multiparous* ewes at equilibrium of the protein balance. Therefore, the mixture of ewes with (low) protein mobilisation and deposition will result in an acceptable efficiency of protein utilisation for milk protein production. Additionally, the percentage of variance accounted for was low in both experiments and the estimated efficiencies were comparable. Therefore, all observations from experiments 1 and 2 could be incorporated in a single dataset resulting in a percentage of variance accounted for of 15% and a milk protein production efficiency for ingested DVE of 0.643 ± 0.01 (Figure 6.5).

Figure 6.5
Protein intake (g DVE) and production of milk protein (g per kg metabolic weight)



It was expected that energy feeding level could increase the efficiency of milk protein production due to less gluconeogenisis from amino acids, but the results of the experiments did not sustain this hypothesis. The results of the present experiments were in agreement with Gonzales et al. (1984), who explained their observation by suggesting that the differences in energy intake were larger than the differences in energy available for milk production due to differences in fat mobilisation. This could also be of importance in our experiments, since fat mobilisation differed between experiments with different energy allowance levels.

Conclusions

For crossbred ewes suckling twin lambs in early lactation at energy intakes of approximately 80% of requirements, a daily DVE intake of 9 g per kg metabolic live weight is sufficient to maintain milk production performance.

The efficiency of utilisation of DVE intake for milk protein production in ewes with a protein balance around equilibrium was comparable between ewes with a low energy intake. However, ewes consuming energy according to requirements displayed an over-all average efficiency for DVE utilisation of 64.3%.

Milk production performance can be increased by feeding a high energy level in combination with a high DVE feeding level, but economic arguments will decide its usefulness in common practice.

The fat to protein ratio in mobilised body tissue of crossbred ewes increased during early lactation in relation to both energy and protein intake.

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Chapter 7

General Discussion

Introduction

Several attempts have been made to improve the economic prospects of sheep farming. A commonly adopted approach in lamb meat production is to cross rams of a meat type breed with prolific ewes to increase litter size while maintaining growing potential and meat quality. However, large litter sizes (>2) often coincide with a reduction in feed intake during late gestation and with low lamb birth weights (Orr and Treacher, 1984 ; Everts, 1990a). Shearing the ewes at 6 to 8 weeks before parturition can diminish these negative effects. In practice this shearing occurs during winter and thus the pregnant ewes have to be housed indoors to protect them from harsh weather conditions. Current Dutch feeding requirements for ewes have been formulated for Texel ewes (meat type) bearing 1 or 2 lambs. Therefore, further investigations are needed to provide general information on the nutritional requirements for prolific ewes and especially for those shorn in midwinter. Since Everts (1990a and 1990b) investigated energy requirements, the present research was focussed on the protein requirements of the prolific Flevolander ewe.

Another reason to investigate protein requirements for sheep, is the introduction of a new Dutch protein evaluation system for ruminants, the DVE/OEB system (Tamminga et al., 1994). This protein evaluation system calculates the amount of protein absorbed from the small intestine (DVE). Since cold exposure is known to affect (ruminal) digestion (Kennedy et al., 1986), the effects of midwinter shearing on DVE evaluation need further investigation. Furthermore, the DVE system was designed primarily for protein evaluation for dairy cows and tables of reference protein values of feedstuffs are therefore based on protocols using dairy cows. These reference values are also used for sheep on the assumption that rumen kinetics are comparable between sheep and cows (Everts, 1992). There are indications (e.g. type of diet, feed intake level) that this assumption is not valid. Finally, DVE requirements for sheep were formulated from literature data (Everts, 1992) and it is necessary to validate these requirements for Crossbred ewes.

Validation of the protein requirements for ewes requires knowledge of DVE evaluation of feedstuffs for sheep, of the (efficiency of) utilisation of the absorbed protein and of the amount of protein needed for maintenance and production. In the present studies several aspects of DVE evaluation for sheep were studied, as well as protein production of the ewe (lamb and milk) and (the efficiency of) protein utilisation by prolific ewes during late gestation and early lactation. In this general discussion the aspects of DVE evaluation for sheep are discussed and combined with the measured protein accretion, milk protein production and utilisation of protein for prolific ewes. It results in a proposal to adapt the DVE requirements for prolific ewes during late gestation and early lactation.

The use of the DVE/OEB system for sheep

According to the DVE/OEB system the DVE value of a feedstuff is based on the amount of undegraded feed protein and the amount of microbial protein that leaves the rumen and enters the small intestine (SI) to be digested. The amount of undegraded feed protein entering the SI is calculated as the complement of the amount of protein that is effectively degraded in the rumen.

Effective rumen degradability (ERD) of feed protein is calculated from a combination of rumen degradation characteristics measured with the nylon bag technique (soluble and undegradable fraction and the degradation rate Kd of the potentially degradable fraction) and an appropriate rumen passage rate (6% per hour for concentrates and 4.5% per hour for roughages).

The amount of microbial protein entering the SI is calculated from the amount of organic matter (OM) that is fermented in the rumen (FOM) in combination with an appropriate efficiency by which rumen microbes convert fermented organic matter into microbial protein (150 g microbial protein per kg FOM). FOM is calculated as the apparently digestible OM (DOM) minus the components from which it is assumed that no energy becomes available for rumen microbes.

Rumen degradation characteristics

Rumen degradation characteristics are susceptible to a number of influences. Some of these influences can be accounted for by standardising the nylon bag technique, although even then substantial differences may be observed between laboratories (Van Straalen, and Tamminga, 1990). Apart from the protocol used, the diet fed is one of the most important sources of variation in results of the nylon bag technique (Lindberg, 1985 ; Nocek, 1988 ; Huntington and Givens, 1995). In sheep not only diet (e.g. roughage:concentrate ratio), but also breed (De Waal, 1995) and midwinter shearing (Kennedy et al., 1976 and 1977 ; Westra and Christopherson, 1976 ; Weston, 1983) can influence rumen degradation kinetics. These differences may be due to differences in rumen pH, (critical) particle size, amount of microbes per unit of volume and microbial activity.

The results of the standardised nylon bag technique as presented in Chapter 2, show differences in rumen degradation characteristics between cows and sheep, between sheep of different breeds and between unshorn and shorn sheep. The results also show that the roughage to concentrate ratio in diets for ewes during gestation, do not affect rumen degradation characteristics.

Species

The differences in degradation characteristics between sheep and dairy cows demonstrate that the table with reference feed protein values should be used with caution for sheep. This incompatibility is probably (partially) induced by differences in the rations fed and differences in the amount of nutrients recycling in the rumen (Lindberg, 1985 ; Nocek, 1988). Nevertheless, the observed higher degradation rates in sheep are in accordance with results of others (Siddons and Paradine, 1983 ; Udèn and Van Soest,1984), which supports the view that differences between rumen kinetics of ewes and dairy cows are not caused by methodological differences alone, but also by differences between species. This is emphasised by the surprising observation that crossbred ewes showed lower degradation rates for grass hay than did dairy cows, while the ewes were adapted to grass hay and the cows were not.

Sheep breeds

Differences observed in rumen degradation characteristics between sheep breeds can not be attributed to differences in live weight, sex, age, physiological state and diet. It suggests that Texel ewes have a more efficient rumen degradation than crossbred ewes, especially for roughages. Possible explanations (differences in chewing and rumination) for these differences have been discussed in Chapter 2 and emphasise the large difference between both sheep breeds. The unexpected low degradation rate of roughage found in crossbred ewes were confirmed by results on degradation rate according to the approach as described in Chapter 3. A lower rumen degradation might be profitable to the animal, because it will result in more undegraded feed protein reaching the SI and thus in a potentially higher DVE yield (unless the microbial protein production is substantially decreased by a smaller amount of FOM or unless the ileal digestibility is reduced).

Cold exposure

The extent of the response to cold exposure is positively related to the effective temperature of the environment relative to the lower critical temperature of the animal (Kennedy et al., 1986). The lower critical temperature is the lower boundary of the thermo neutral zone (the range in which the animal maintains body core temperature without expending additional energy above its minimal maintenance requirement). Rumen retention time in closely shorn ewes exposed to ambient temperatures of 20-25 °C was considerably higher than the retention time in closely shorn ewes exposed to temperatures of 0-5 °C (Kennedy et al., 1986). It is commonly believed that this (by cold exposure induced) increase in rumen outflow rate (Kennedy et al., 1986 ; Ngongoni et al., 1987) will lead to a decrease in effective rumen degradation. Therefore, our observation that midwinter shearing enhanced rumen degradation in general and for crude protein (CP) with statistically significance, is

surprising. It is not very likely that this is due to the comparison of unshorn ewes with shorn ewes at comparable temperatures (on average 3.7 °C), because shearing results in an approximately 20-25 °C increase in lower critical temperature which is comparable to that of the shorn ewes at different temperatures as mentioned by Kennedy et al. (1986). Results from nylon bag studies can not explain the observed differences, but cold exposure changes the relative amounts of microbes (including protozoa) in the rumen, the efficiency of microbial production and the neural and endocrine regulation of the processes of the digestive tract (Kennedy et al.,1986). Apparently, these changes also result in a higher rumen degradation rate.

Roughage to concentrate ratio

Generally an increase in the amount of concentrates in the diet results in a decrease in degradation rate of cell walls and protein of roughages (Siddons and Paradine, 1981 ; Weakly et al., 1983 ; Zhao et al.,1993 ; De Waal, 1995 ; Archimède et al., 1996). However, the differences in roughage to concentrate ratio in diets for ewes during gestation are probably not large enough to alter rumen degradation characteristics. This is especially the case when high quality roughages are fed (Cronjé, 1992). Therefore, the roughage to concentrate ratio in diets for ewes will not be subject to further discussion.

Effective rumen degradability (ERD)

The impact of differences in rumen degradation characteristics (Chapter 2) on feed evaluation is not clear unless rumen passage rate (Kp) is taken into account. Most protein evaluation systems (including the DVE/OEB system) assume constant Kp's for protein in roughages and concentrates. This assumption is suitable for common protein evaluation, but may be questionable when the animals are exposed to cold (Kennedy et al., 1986). Besides, it may be questionable to use the same Kp for sheep and dairy cows, because of differences in the amount of nutrients recycling in the rumen (Nocek, 1988 ; Lindberg, 1985). In Chapter 3 the available Kp estimation techniques are discussed and it is concluded that estimation of ERD from changes with time in the rumen volume of nutrients partitioned into their rumen degraded and rumen undegraded components is an alternative to *in vivo* measurement of ERD or to the use of Kp. By using changes in rumen volume with time, ERD is studied without using feed characteristics (e.g. Kd, S and D). A (large) disadvantage of this approach is that it is impossible to distinguish between the different feedstuffs in the diet.

Sheep breeds

The results of the estimation of ERD from changes with time in the rumen volume as described in Chapter 3 relate to the comparison of unshorn and shorn crossbred ewes. The

same approach can be used to estimate rumen degradation kinetics for Texel ewes and to compare them to the results for crossbred ewes in Chapter 3 (Table 7.1). Due to the small numbers of animals used (3 Texel ewes), the results are not presented in Chapter 3. It is nevertheless worthwhile to have some indication of possible breed differences in rumen degradation kinetics and to relate the results of this comparison to the results of the comparison of degradation rates between breeds (Chapter 2).

Table 7.1
Rumen degradation kinetics of crude protein (CP) and of crude protein free organic matter (OM-CP) for Texel (Tex) and Crossbred ewes (Cross).

	CP		OM-CP	
	Tex	Cross	Tex	Cross
Intake (g/day)	243	198	835	801
Rumen pool size (g)	219	214	588	587
Rumen degradation rate (%/h)	5.4	3.8	4.7	3.7
Rumen passage rate (%/h)	3.5	3.1	3.8	4.5
Effective Rumen Degradability (%)	55.6	49.0	47.9	35.5

The degradation rate of nutrients is higher in the rumen of Texel ewes than of crossbred ewes, which is in agreement with the results of Chapter 2. Combined with rumen passage rate this results in a higher effective rumen degradation of N (less undegraded feed entering the SI) and of OM-CP (indicating a larger amount of microbial protein entering the SI) in Texel ewes. The absolute value of the presented figures for both degradation and passage rate are discussed in Chapter 3 and may differ from the results of the nylon bag technique (Kd) and marker techniques (Kp), but the presented figures are suitable for comparison and the results on ERD are reliable (Aitchison et al., 1986a). The higher passage rate of OM-CP in crossbred ewes may have resulted in a higher efficiency of microbial protein synthesis (less microbial turnover due to a lower rumen residence time), but the experiments do not provide enough information about rumen fluid passage rate to sustain this hypothesis. However, breeds seem to differ in rumen pool size in relation to intake. Texel ewes ingested more CP and OM-CP, but had nevertheless comparable rumen pool sizes. For crossbred ewes the rumen pool size of crude protein was even larger than the daily intake, which might be an indication for a larger microbial mass. This hypothesis is sustained by contradicting results for Kp of CP and OM-CP in the comparison between breeds. The higher Kp for OM-CP in crossbreds will lead to a higher outflow of microbial protein since most microbial protein is attached to the fibrous components of the rumen content and the microbial mass in the rumen content will be smaller in crossbred ewes. However, the

observed microbial mass in the rumen at comparable rumen pool sizes does not differ significantly between breeds (Table 7.2), although Texel ewes seemed to have a larger rumen microbial mass in the first 4 hours after feeding. This observation can only be explained by a higher efficiency of microbial growth in g / kg FOM in crossbred ewes. The higher microbial growth may lead to a higher microbial contamination of the nylon bags used to estimate the undegraded fraction of rumen contents and thus to a decrease in the calculated Kp (of the Aitchison model) for crude protein in crossbred ewes (as shown in Table 7.1).

Table 7.2
Microbial crude protein (grams) in rumen contents at 1, 2 ,4, 8 and 12 hours after feeding. All averages are corrected for DM intake.

	Texel	Crossbred	S.E.D.
hours after feeding			
1	105.1	101.2	7.9
2	108.8	103.1	4.0
4	103.0	102.6	8.7
8	90.2	89.8	9.3
12	79.4	82.5	6.7

It seems therefore reasonable to conclude that crossbred ewes yield more DVE from their feed than Texel ewes, since the amount of both undegraded feed protein (lower ERD) and microbial protein (larger Kp OM-CP) appears to be larger in crossbred ewes.

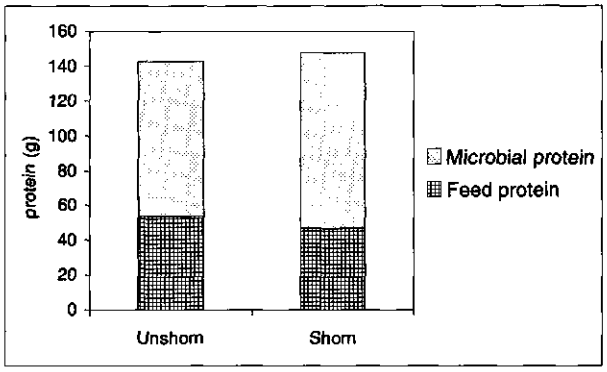
Cold exposure

The results of the feeding trials with shorn and unshorn ewes (Chapter 5) show a positive effect of midwinter shearing on lamb birth weight and suggest a higher protein supply of the shorn ewes at equal feed intake. This observation is in agreement with literature, where cold exposure is related to an increased supply of undegraded feed protein due to an increased passage rate. However, the expected increase in rumen passage rate (Kennedy et al., 1986 ; Ngongoni et al, 1987) was not observed in the present experiments (Chapter 3). It has already been mentioned that the results of the model used to calculate Kp are reliable for comparison of treatments (Aitchison et al., 1986a) and the absence of an effect of cold exposure on rumen passage rate is in agreement with the results of the marker technique on unchopped grass diets (Kennedy et al., 1982 and 1985). The contradicting results of the marker technique used to investigate the effect of cold exposure on Kp could be the result of diet preparation since increases in Kp due to cold exposure are reported on diets that

were milled and compounded (Kennedy et al., 1986 ; Ngongoni et al, 1987). However, protein evaluation is based on ERD, a combination of Kp and Kd, and the results of the method used in Chapter 3 to estimate ERD are reliable (Aitchison, 1986a). It is clear that cold exposure increases rumen degradation rate and ERD. A higher ERD for dietary protein will result in a smaller amount of undegraded feed protein reaching the SI of shorn ewes. Therefore, the only option left to explain the higher protein supply of shorn ewes is an increased supply of microbial protein. An indication of increased microbial protein supply is provided by the observed smaller rumen pool sizes in shorn ewes (Chapter 3). A smaller rumen pool size has been associated with a larger ruminal outflow of microbial protein (Chen et al., 1992 ; Meissner et al., 1996 ; Ranilla et al., 1998) and with an increased efficiency of microbial growth (Hespell and Bryant, 1979). It is also in agreement with a smaller size of the rumen fluid pool as a result of an increased fluid passage rate (including rumen microbes) in cold exposed ewes (Kennedy et al., 1986).

The results of Chapter 3 allow calculation of the amount of protein reaching the SI, which appears to be comparable between unshorn and shorn ewes (Figure 7.1).

Figure 7.1
The amount of protein reaching the small intestine (at equal intake) of unshorn and shorn crossbred ewes



However, the amount of microbial protein reaching the SI is calculated under the assumption that the efficiency of microbial growth is identical in the rumen of unshorn and shorn ewes. This assumption is probably not valid, since cold exposure increases the efficiency of microbial growth in the rumen of sheep substantially (Kennedy and Milligan, 1978 ; Kennedy et al., 1986). Kennedy and Milligan (1978) found increases of 13-42% in microbial mass production on brome grass diets. In contrast, Kennedy et al. (1982) did not find a significant increase in efficiency of microbial protein synthesis due to cold exposure,

but concluded that this was due to a different treatment of the diets and stated that the results of Kennedy and Milligan (1978) were probably a better reflection of microbial protein synthesis. The efficiency of microbial growth was not measured in the present research, but under the assumption that winter shearing increases the efficiency of microbial growth within the range observed by Kennedy and Milligan (1978), the results of Figure 7.1 can be recalculated (Table 7.3). Table 7.3 also provides the amount of protein digested in the SI, since cold exposure increases the amount of NAN digested in the intestine (Kennedy et al., 1986). The digestibilities used to calculate the amount of intestinal digested undegraded feed protein (90%) and microbial protein (64%) are according with the DVE/OEB system.

Table 7.3
Amounts of protein entering and digested in the small intestine (SI) of unshorn and shorn crossbred ewes. Calculations based on the results of the present study and for microbial protein based on the efficiency of microbial growth of the DVE/OEB system (150 g/ kg fermented OM) and on an assumed increase of the efficiency of microbial growth of 13% and 42% (respectively 170 and 213 g/ kg fermented OM).

	Undegraded feed protein		Microbial crude protein		Total protein
	Entering SI	Digested in SI	Entering SI	Digested in SI	digested in SI
Unshorn (150)	54.0	48.6	89.0	56.7	105.3
Shorn (150)	47.0	42.3	101.0	64.4	106.7
Shorn (170)	47.0	42.3	114.5	73.0	115.3
Shorn (213)	47.0	42.3	143.4	91.4	133.7

Given the assumed range of increase in efficiency of microbial growth due to midwinter shearing, the amount of microbial protein entering the SI increases from 101 grams to 114 - 143 grams daily. The total amount of protein digested in the SI of shorn ewes increases within the range from 9 - 27%, which would lead to a comparable increase in the amount of DVE available to the cold exposed ewe. However, it must be emphasised that this is a theoretical increase, since the present research does not supply data on the efficiency of microbial growth.

DVE evaluation of feedstuffs for sheep

DVE evaluation according to the DVE/OEB system is designed for dairy cows. The results of the present research show that this approach needs to be adapted when used for sheep ; sheep differ from cows in degradation rate (Chapter 2) and the feeding trials (Chapters 5 and 6) suggest that DVE was underestimated for sheep. Underestimation of DVE can be

due to underestimation of the amount of undegraded feed protein and/or of the amount of microbial protein reaching the SI. Within the DVE/OEB system the amount of protein reaching the SI of sheep can be corrected by adapting the ERD for protein and by adapting FOM and/or changing the efficiency of microbial protein synthesis. Both ways of adapting DVE calculation will be discussed.

Adapting the calculation of the amount of rumen undegraded feed protein

The calculated amount of undegraded feed protein reaching the SI can be adjusted by adapting the ERD for protein, which is possible by adjusting Kd and Kp. A direct measurement of Kd in sheep and comparison of Kd between sheep and cows is discussed in Chapter 2, but a direct comparison of Kp for sheep and cows is not available. However, for sheep Chapter 2 provides the Kd, the potentially degradable-, the undegradable- and the soluble fraction of the feeds used and Chapter 3 provides ERD. This information can be used to calculate Kp of the diet by combining it with the common way of calculating the effective rumen degradability of feed protein. Under the assumption that the Kp-roughage to Kp-concentrate ratio for sheep is comparable to that of cows (=1.33), the Kp for roughage and concentrates can be estimated separately (Table 7.4).

Table 7.4
Calculated rumen passage rate (Kp) of feed protein for Texel ewes and crossbred ewes

	Kp roughage	Kp concentrate
Texel	3.58	4.76
Unshorn crossbred	3.60	4.79
Shorn crossbred	3.63	4.83

It seems that passage rate does not differ between the sheep breeds under consideration nor between unshorn and shorn crossbred ewes. On average the Kp in sheep for roughage and concentrates is 3.6 and 4.8 % per hour respectively. These Kp's are in agreement with Kp measured *in vivo* (marker technique) with sheep fed rations of chopped hay of perennial ryegrass supplemented with maize starch at a high feeding level (Aitchison et al., 1986b), which is comparable to the ration and feeding level of the present experiments. Compared to Kp's for cows used in the DVE/OEB system (4.5 and 6.0 % per hour for roughage and concentrates respectively) sheep have lower rumen passage rates.

Differences in Kp between species may be due to differences in (critical) particle size, in particle size reduction and in rumen volume in relation to feed intake. Within species, body weight (BW) is indicative for ruminal volume and Kp is related to BW and to dry matter

intake (DMI) in a way that Kp increases with increasing DMI / kg BW (Chen et al., 1992). However, gastro-intestinal capacity is proportional to metabolic size and therefore proportionally greater in smaller animals (Van Soest,1994). Indeed, the observed contents of the gastro-intestinal tract (as a % of BW) were approximately 15% for cows and 20% for sheep (Van Soest,1994). Therefore, BW can not be used for the relationship between intake and rumen volume. For this comparison between species DMI / kg $W^{0.75}$ has to be used (Table 7.5).

Table 7.5
Calculated dry matter intake (DMI) in grams per kg metabolic weight ($W^{0.75}$) for ewes and for dairy cows at comparable energy feeding levels (times maintenance).

	Feeding level	g DMI / kg $W^{0.75}$
Ewe late gestation	1.7	43
Ewe suckling twins	3.6	85
Dairy cow 8 kg FPCM	1.7	75
Dairy cow 30 kg FPCM	3.6	154

Table 7.5 shows the DMI of ewes as observed in the present study for late gestation and early lactation (respectively Chapter 5 and 6) and for cows of 650 kg live weight at a comparable energy feeding level as sheep, according to Dutch standards (CVB, 2000). According to the DMI / kg $W^{0.75}$ ewes ingest at comparable energy feeding levels approximately 56% of the amount that is ingested by cows. This will most likely result in lower passage rates in sheep than in cows, especially for ewes during late gestation, which is in agreement with Table 7.4. For ewes in early lactation DMI / kg $W^{0.75}$ is comparable to that of low producing cows, which suggests that for this category of ewe the Kp of the DVE/OEB system could be valid. Consequently, with regard to the aspect of passage rate, the amount of undegraded feed protein reaching the SI in ewes during late gestation will be overestimated (Kp overestimated), but probably not in ewes during early lactation.

Adapting the calculated amount of microbial protein synthesis

The calculated amount of microbial protein can be changed by adapting the calculation of FOM or by adapting the efficiency of microbial protein synthesis. FOM is calculated as DOM minus its components from which it is assumed that no energy becomes available for rumen microbes. DOM is estimated *in vitro* (Tilley and Terry, 1963). This approach is identical for sheep and cows and is therefore not suitable for adaptation. The efficiency of microbial protein synthesis in the DVE/OEB system is adapted from Russel et al. (1992)

who observed a close relationship between predicted and measured bacterial CP flow in the duodenum. However, the assumed efficiency of microbial protein synthesis in the DVE/OEB system of 150 g / kg FOM might underestimate the amount of microbial protein reaching the SI of sheep. According to literature the efficiency of microbial protein synthesis in sheep ranges from 176 to 252 g / kg FOM (Kennedy and Milligan, 1978 ; Kennedy et al., 1982). Combined with the results of Table 7.3 it shows that the DVE/OEB system might underestimate the amount of protein digested in the SI of sheep by 9 - 31%.

Protein requirements and utilisation of DVE in sheep

The protein requirement of sheep can be presented as the sum of the requirements for maintenance and for production. To estimate DVE requirement, the amount of protein used for maintenance and production can be measured and combined with the efficiency by which DVE is used for maintenance and production. The protein requirement for maintenance is well documented and is expressed per kg metabolic live weight ($W^{0.75}$). The Dutch maintenance requirement for ewes is 1.5 g DVE / kg $W^{0.75}$ (Everts, 1992) and includes the protein required for wool growth, but excludes the protein lost with metabolic faecal N (MFN). Chapter 4 provides a maintenance requirement for crossbred ewes of 511 mg truly digestible NAN / kg $W^{0.75}$ or 3.2 g truly digestible protein (DVE) / kg $W^{0.75}$. This approximates the 2.95 g of metabolisable protein / kg $W^{0.75}$ for a 80 kg live weight ewe according to AFRC (1995). However, this maintenance requirement excludes wool growth, but includes replenishment of the amount of protein lost with MFN. The DVE/OEB system corrects for replenishment of MFN based on the relationship between MFN and indigestible dry matter intake. Therefore, the requirement for MFN is subtracted from the maintenance requirement of ruminants when used in the DVE/OEB system. According to the NRC (1985), the amount of MFN is approximately 1.3 g / kg $W^{0.75}$. Assuming an efficiency of 0.67 by which DVE is used to produce MFN, this means that in the present experiments 1.95 g DVE / kg $W^{0.75}$ of the maintenance requirement originates from MFN. Therefore this gives an amount of 3.2 – 1.95 = 1.25 g DVE / kg $W^{0.75}$ needed for maintenance. The requirement for wool growth is 0.4 g DVE / kg $W^{0.75}$ (Everts, 1992), which brings the total maintenance requirement to 1.65 g DVE / kg $W^{0.75}$.

Protein accretion and utilisation during late gestation

Protein accretion in multiparous ewes during late gestation mainly concerns growth of wool, the udder, the uterus and the products of conception (foetuses, placenta and fluids). The growth of wool is commonly defined as part of the maintenance requirement and the growth of the udder and uterus largely depends on redistribution of maternal protein from skeletal muscle (McNeill et al., 1997). The maternal protein change (calculated as the ewes N balance minus protein accretion in the products of conception) will therefore be negative for ewes carrying large litters.

The protein accretion in foetuses, placenta and fluids during various stages of gestation can be estimated with equations provided by McDonald et al. (1979). In Chapter 4, genotype related differences in protein accretion in foetuses are accounted for by adapting the Gompertz curves to the analysed N content of lambs at birth (including the actual days in conception and number of foetuses). These adapted equations are used to estimate the daily protein accretion in the products of conception of crossbred ewes during late gestation (Table 7.6). The average observed litter weight in the present experiments is 7.8, 10.1 and 12.2 kg for twins, triplets and quadruplets respectively.

Table 7.6
The average protein accretion (g/d) in foetuses, placenta and fluids of crossbred ewes during several periods in late gestation.

Litter size	2	3	4
Days in gestation			
100-120	17.8	23.1	27.2
120-130	26.0	33.3	38.8
130-145	31.6	40.1	46.3

The AFRC (1995) provides efficiencies for conceptus growth and for growth in ewes of 0.85 and 0.59 respectively. This is in fairly good agreement with the efficiencies in the present research (Chapter 4) for ewes with a positive maternal protein change. However, under the assumption that maternal protein change excludes the protein accretion in the udder and uterus (due to redistribution of maternal protein), most ewes will have a negative maternal protein change. Therefore, the efficiency for the protein accretion in conceptus growth of 0.54 for ewes with a negative maternal protein change is preferred in the present investigations.

Protein accretion and utilisation during early lactation

Chapter 6 provides both the average daily protein excretion in milk of *multiparous* crossbred ewes during the first six weeks of lactation (in lactation week 1 to 6 respectively 137, 146, 140, 129, 120 and 109 grams daily) and the efficiency by which protein intake above maintenance is used to produce milk protein (on average 64.4%). The results of the experiments with different energy intake levels show that common Dutch feeding practice enables the ewes to produce sufficient milk (protein) for good lamb performance without decreasing the efficiency of milk protein production. It seems that milk (protein) production can be increased by feeding high levels of both protein and energy. However, such feeding strategies are not likely to become common practice

due to high costs and due to the fact that lamb performance was comparable to the other feeding strategies. For crossbred ewes (75 kg live weight) during the first 6 weeks of lactation, a feeding level of approximately 80% of energy requirements and a protein intake of approximately 9 grams DVE / kg $W^{0.75}$ seem to be sufficient to maintain production.

Implications for the feeding strategy of crossbred ewes during late gestation and early lactation

Feed evaluation

Protein feeding strategies based on the DVE/OEB system should preferably use DVE values as calculated from the reference tables of protein values of feedstuffs. The results of the present investigations show that this approach may lead to underestimation of the DVE value of feeds for sheep. Therefore, a comparison is made between the DVE value of the ration (as ingested on average in the present experiments) according to the tables of reference and the DVE value for Texel ewes and for unshorn and shorn crossbred ewes (Table 7.7). All DVE values are calculated according to the DVE/OEB system using the results for the rations Kd, D-, U- and S-fraction as observed in the experiments and Kp from Table 7.4. The used efficiency of microbial protein synthesis is based on this General Discussion, being higher in crossbred ewes than in Texel ewes and higher in shorn than in unshorn ewes. The range of efficiencies of microbial growth for sheep is 160-250 g

Table 7.7
DVE value per kg DM of the grass hay / concentrate diet as ingested in the present experiments. DVE calculated according to the reference tables of DVE values for feeds and the present experiments for Texel ewes and for unshorn and shorn crossbred ewes.

	Micr.eff.[1)]	DVBE[2)]	DVME[2)]	DVMFE[2)]	DVE[2)]
Reference value	--	--	--	--	105
Texel ewes	160	66	56	20	102
Crossbred ewes, unshorn	200	83	56	20	118
Crossbred ewes, shorn	250	73	73	20	126

[1)] Micr.eff. = efficiency of microbial synthesis in g / kg FOM

[2)] DVBE = amount of digestible undegraded feed protein, DVME = amount of digestible microbial protein, DVMFE = endogenous protein losses resulting from digestion, DVE = intestinal digestible protein

microbial protein per kg FOM (Kennedy and Milligan, 1978), in which the lowest levels were measured with sheep in common environmental conditions and the highest values in cold exposed sheep. Therefore, it is assumed that the lowest value is valid for Texel ewes and the highest for shorn crossbred ewes. Unshorn crossbred ewes showed a comparable amount of microbial protein at lower FOM than Texel ewes (this General Discussion), which indicates a higher efficiency of microbial growth. The efficiency of microbial growth in unshorn crossbred ewes is calculated to result in the same microbial yield as Texel ewes, which results in a microbial protein yield of 200 g per kg FOM. According to Table 7.7 the Texel ewes, unshorn crossbred ewes and shorn crossbred ewes yielded respectively 97%, 112% and 120 % of the DVE (per kg DM) of the reference DVE value of the ration under consideration. These percentages can be used to correct the minimum DVE ingestion levels to maintain production as observed in Chapters 5 and 6. As stated in this General Discussion the DVE values for ewes in early lactation are likely to differ less from the reference DVE value than for gestating ewes. This is due to the fact that Kp of lactating ewes will resemble Kp of dairy cows and a similar case can be argued for Kd.

DVE requirements during late gestation

The daily amount of DVE necessary for production and maintenance of crossbred ewes during late gestation (Table 7.8) comprises DVE needed for maintenance and for growth of the products of conception. The daily protein requirement for maintenance is estimated to be 1.65 g DVE / kg $W^{0.75}$. For an average ewe of 80 kg live weight during gestation this implies 44 g DVE daily. The daily protein accretion in foetuses, placenta and fluids results from dividing the information in Table 7.6 by 0.54, being the efficiency by which DVE is utilised for this protein accretion.

Table 7.8
Experimentally determined amount of DVE (gram / day) necessary for production, maintenance and wool growth of crossbred ewes of 80 kg live weight during several periods in late gestation.

Litter size	2	3	4
Days in gestation			
100-120	77.0	86.8	94.4
120-130	92.2	105.7	115.8
130-140	102.4	118.2	129.6
140-147	109.0	125.9	137.4

According to table 7.8 the average required amount of DVE during the last 47 days of gestation is 90, 103 and 113 g DVE daily for ewes bearing twin, triplets and quadruplets

respectively. From Chapter 5, where DVE values according to the reference table (= *standard* DVE) are used, it is clear that crossbred ewes bearing on average 3.3 lambs, are able to maintain maternal body weight and reproductive performance on 3.6 g *standard* DVE / kg $W^{0.75}$ during late gestation. When corrected for DVE evaluation differences between *standard* DVE and calculated *experimental* DVE for unshorn crossbred ewes (12%) this means a daily average of 108 g *experimental* DVE from 100 days of gestation until parturition for unshorn crossbred ewes of 80 kg. This average is in agreement with the average for triplets derived from table 7.8. For shorn crossbred ewes during late gestation and bearing 3.5 lambs, the ingestion of a to unshorn ewes comparable intake of *standard* DVE (4.6 g / kg $W^{0.75}$) resulted in an increased sum of birth weights (Chapter 5). When corrected for DVE evaluation differences between *standard* and *experimental* DVE values for unshorn and shorn crossbred ewes (12 and 20% respectively) intake was not comparable but shorn ewes ingested on average 25 g *experimental* DVE / day more from 100 days of gestation until parturition than unshorn ewes. This might explain the increased lamb birth weight. Comparison of the average ingestion of shorn ewes with Table 7.8 is not valid since the sum of birth weights is increased due to midwinter shearing.

DVE requirements during early lactation

The daily amount of DVE necessary for production and maintenance of crossbred ewes during early lactation (Table 7.9) comprises of DVE needed for maintenance and for milk protein production.

Table 7.9
Experimentally determined amount of DVE (gram / day) necessary for production, maintenance and wool growth of crossbred ewes suckling twins during the first six weeks of lactation.

Week in lactation	1	2	3	4	5	6
DVE (g daily)	255	269	259	242	228	211

The required daily amount of protein for maintenance is estimated to be 1.65 g DVE / kg $W^{0.75}$. For an average ewe of 75 kg live weight during lactation this implies 42 g DVE daily. The daily protein excretion in milk divided by the average measured efficiency of 64.3% (Chapter 6) results in the required daily amount of DVE for crossbred ewes during late lactation. From Chapter 6 it is clear that these crossbred ewes maintain milk production performance at an ingestion level of 8.7 g *standard* DVE / kg $W^{0.75}$ during early lactation. A correction for feed evaluation is not available for lactating ewes, but will be smaller than

the 12% for unshorn gestating ewes due to a higher resemblance with dairy cows in rumen kinetics. Without correction for feed evaluation the requirements for crossbred ewes of 75 kg live weight are 222 g *experimental* DVE daily during the first six weeks of lactation. According to table 7.9 these required amount of DVE is on average 244 g daily. The difference of 22 g DVE daily must be due to protein mobilisation or to a underestimation of the DVE value. The average protein mobilisation of 2 g protein yields approximately 1.5 g DVE daily for maintenance and production. The remaining calculated difference of 20 g DVE daily can be accounted for by a 8% correction for the difference between *experimental* DVE and the *standard* DVE.

Conclusions

Rumen degradation characteristics and rumen passage kinetics differ between prolific ewes during late gestation and dairy cows producing 15 kg FPCM or more, in such a way that prolific ewes yield more DVE from the same amount of feed. Rumen degradation characteristics (but not rumen passage kinetics) are also affected by midwinter shearing of pregnant ewes resulting in a higher DVE yield from the same amount of feed. The concentrate/roughage ratios as observed in common sheep husbandry do not affect DVE evaluation of feedstuffs.

The calculated differences in DVE evaluation are due to differences in the calculated effective rumen degradability of dietary protein and organic matter as well as to (assumed) differences in the efficiency of microbial protein synthesis. Both are higher in sheep than in cows and both are increased by midwinter shearing. The contribution of the amount of microbial protein reaching the small intestine of crossbred ewes to the calculated DVE value is large (60-70%) and emphasises the importance of how the efficiency of microbial protein synthesis is estimated. Further research is needed to quantify the amount of microbial protein synthesised per kg fermented organic matter in Texel ewes and in unshorn and shorn crossbred ewes.

Few observations were made for Texel ewes, but Texel ewes resemble dairy cows and differ from prolific ewes in DVE evaluation. Texel ewes have a higher effective rumen degradability of dietary protein and organic matter than crossbred ewes. The present experiments do not justify any adaptations in DVE evaluation or DVE requirements for Texel ewes.

For crossbred ewes during late gestation and early lactation the present experiments provide the amount of protein produced in offspring and milk as well as the efficiencies by which the ingested DVE is used for this production. The DVE requirements calculated from the measured protein production and efficiency of utilisation are in agreement with the DVE requirements derived from feeding trials for both late gestation and early lactation.

Practical implications and proposal for adaptation of DVE requirements for crossbred ewes

The results of the present investigations show that DVE evaluation and DVE requirements for crossbred ewes require modification. Because of lack of sufficient data, for Texel ewes modification is not justified.

DVE evaluation for crossbred ewes

The most pragmatic approach for DVE evaluation for crossbred ewes is according to the reference table with DVE values of feedstuffs. Differences between *standard* DVE values and *experimentally determined* DVE values have to be corrected by adapting the DVE requirements.

DVE requirements for crossbred ewes during late pregnancy
The table with the required *standard* DVE ingestion for unshorn and shorn crossbred ewes during the last 2 months of pregnancy carrying twins, triplets or quadruplets (Table 7.10) can be adapted to other live weights by changing the maintenance requirement by 1.65 g DVE / kg $W^{0.75}$ daily. The required ingestion levels have to be corrected for feed leftovers in order to calculate the daily allowance.

Table 7.10
Required ingestion of standard DVE (gram / day), including DVE for maintenance and wool growth, of crossbred ewes carrying different litter sizes during the last two months of gestation.

Live weight	Twins[1)]	Triplets[1)]	Quadruplets[1)]
60	77	89	98
70	80	92	101
80	82	94	103
90	85	97	106
100	87	99	108
110	90	102	111

[1)] Average sum of birth weights of 7.8, 10.1 and 12.2 kg respectively

DVE requirements for crossbred ewes during early lactation

Daily DVE requirements of 250 and 210 g DVE for *multiparous* ewes suckling 2 lambs during the first and second month of lactation respectively can be adjusted to 240 and 200 g *standard* DVE daily for crossbred ewes.

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Summary

There are two reasons for reconsidering ewe feeding strategies. First, because of the introduction of crossbred ewes into sheep husbandry and secondly the introduction of a new protein evaluation system for ruminants, the DVE/OEB system. Crossbred ewes were introduced into sheep husbandry to increase annual lamb meat production per ewe by increasing litter size. By mating these crossbred ewes with rams of the Texel breed, the meat producing qualities of Texel sheep can be combined with the prolificacy of crossbred ewes. However, current sheep feeding strategies are based on ewes with small litters and may not be adequate for crossbreds. Since energy requirements have already been investigated, the present research focussed on the protein requirements of the crossbred ewe. The DVE/OEB system resulted from the growing concern of our society about the effects of intensive animal husbandry systems on the environment. It was developed as a tool to balance protein feeding with protein requirements by describing protein digestion and metabolism in more detail. However, the DVE/OEB system was designed primarily for protein evaluation for dairy cows and reference protein values of feedstuffs are therefore based on protocols using dairy cows. These reference values are also used for sheep on the assumption that rumen kinetics are comparable between sheep and cows. There are indications (e.g. type of diet, feed intake level) that this assumption is not valid.

During late pregnancy and early lactation it is difficult for the ewe to sustain nutritional supply and demand. The protein requirements of ewes depend on the amount of protein needed for maintenance and production (protein accretion in foetuses and milk protein production), in combination with the efficiency by which feed protein is utilised for accretion in foetuses or for milk protein production. During late pregnancy, ewes with more than one foetus often have a reduced capacity for feed intake, which may result in low lamb birth weights. Low lamb birth weights impinge on the economics of sheep farming. Lamb birth weight can be increased by raising protein ingestion and by winter shearing the pregnant ewes at 6 to 8 weeks before parturition. Both ways have been investigated in this thesis. The protein (and energy) intake of lambs during the period of early lactation is essential for lamb growth performance until slaughtering. Therefore, milk protein production and the efficiency by which feed protein is utilised for milk protein production were also investigated.

The DVE value of a feedstuff is based on the amount of rumen undegraded feed protein and the amount of microbial protein that is digested in the small intestine. The amount of rumen undegraded feed protein is calculated as the complement of the amount of effectively degraded protein in the rumen. The effective rumen degradability is calculated from the soluble and undegradable fraction, the degradation rate of the potentially degradable fraction and the rumen passage rate. The amount of microbial protein is calculated from the combination of the amount of organic matter that is fermented in the rumen and the efficiency of microbial protein synthesis. Rumen degradation characteristics are susceptible to a number of influences. The diet fed is one of the most important sources of variation and it is therefore likely that cows and sheep differ in rumen degradation characteristics. In

sheep not only diet, but also breed and midwinter shearing can influence rumen degradation kinetics.

The aim of this thesis was to investigate the protein requirements of crossbred ewes and to validate the use of the current DVE recommendations for crossbred ewes. To this aim knowledge concerning DVE values for sheep and the use of DVE reference values of feedstuffs had to be validated for sheep. Comparison of rumen degradation kinetics between dairy cows and sheep, between Texel and crossbred ewes and between unshorn and shorn crossbred ewes was also required. The effect of winter shearing on the effective rumen degradability of protein and organic matter as well as on DVE evaluation for crossbred ewes had to be investigated. Knowledge was also required concerning the (efficiency of) utilisation of the absorbed protein and the amount of protein needed for maintenance and production during late gestation and early lactation. For ewes during late pregnancy this knowledge should be available for winter shorn ewes as well as for unshorn ewes. Finally, the protein requirements had to be validated in feeding trials with crossbred ewes during late gestation and early lactation.

In Chapter 2 the results of the standardised nylon bag technique are presented for the rumen degradation rate (Kd) and the rumen undegradable residue (U) of dry matter (DM) and crude protein (CP) in sheep and dairy cows. They show that sheep degraded more DM and CP in the rumen than cows. This difference was largest between cows and Texel ewes, since Texel ewes degraded more nutrients in the rumen than crossbreds. Winter shearing increased the amount of rumen degraded CP in crossbred ewes, but dietary roughage to concentrate ratio did not affect rumen degradation characteristics. The experiments performed support the view that reference protein values of feedstuffs may not be valid for sheep, since rumen degradation kinetics were different in cows and ewes. It would seem that a different approach to feed protein evaluation is required for cows, sheep breeds and (un)shorn ewes.

In Chapter 3 the effective rumen degradability of dietary protein and organic matter was estimated from changes in rumen volume of both degraded and undegraded fractions with time. The obtained values were compared to the effective rumen degradability estimated with the standard method of in situ feed characteristics and an assumed rumen passage rate. The results were also used to study the effects of winter shearing of crossbred ewes. Protein evaluation based on changes in rumen digesta volume with time displayed more variation and a 10% higher DVE value than the commonly used effective rumen degradability. Winter shearing of crossbred ewes increased the extent of degradation in the rumen. These differences did not result in different DVE yields between unshorn and shorn ewes upon assumption of an unchanged efficiency of microbial protein growth during cold exposure. In conclusion the commonly used effective rumen degradability is more suitable for routine techniques than the one based on time related changes in rumen digesta volume. However, it should be taken into consideration that the commonly used effective rumen degradability

may result in an underestimation of the DVE value for crossbred ewes. Protein evaluation should take account of differences in efficiency of microbial protein synthesis between unshorn and shorn ewes to avoid additional underestimation of the DVE value.

In Chapter 4 the protein utilisation in pregnant ewes and protein utilisation for foetal growth were studied using nitrogen (N) balances of pregnant ewes and chemical analyses of new born lambs. The N retention in pregnant ewes and the urinary N output increased with increasing N intake. Concomitantly N mobilisation from the maternal body decreased. As a result the N accretion in foetal growth was not affected by N intake. The chemical composition of lambs from unshorn ewes displayed increasing DM and protein contents with increasing N intake. The overall efficiency by which the ingested N is retained was 66% in crossbred ewes in late gestation. The maintenance requirement of these ewes was 444 mg apparently digested N per kg metabolic live weight. In ewes with a small positive or a negative N accretion, the efficiency by which the ingested N is retained was 54% for foetal growth and 91% for maternal mobilisation. It was concluded that the current feeding strategy is applicable for crossbred ewes with regard to the used efficiencies for protein utilisation in late gestation.

In Chapter 5 the effect of different protein feeding levels and of winter shearing during late pregnancy on feed intake and reproductive performance was investigated in feeding trials with crossbred ewes. The protein feeding levels involved supplied 80%, 100% and 120% of expected requirements, while energy was supplied according to the expected requirements. The effects of winter shearing were investigated with and without *ad libitum* roughage (grass hay) intake. Feed intake remained stable in unshorn ewes during late gestation and was sufficient to meet the expected energy requirements, but winter shearing increased feed intake. Reproductive performance (i.c. lamb birth weight) was not affected by protein feeding level, which indicated that all protein feeding levels supplied sufficient protein to the ewes. Winter shearing increased reproductive performance, although protein intake was comparable to the high protein feeding level. This was explained in three ways : reproductive performance can be increased by a higher supply of both energy and protein, the protein supply of shorn ewes might have been underestimated or shearing altered the nutrient partitioning between mother and foetus. Treatments did not affect the change in body condition score and wool growth, but shorn ewes lost more body weight during gestation. This was attributed to a higher deposition in lambs and higher energy requirements for maintenance of the ewes. It was concluded that a stable feed intake during pregnancy can be achieved by feeding a concentrate / roughage diet with increasing concentrate allowance, that the current Dutch protein requirements could be reduced for crossbred ewes and that midwinter shearing was effective in improving lamb birth weight.

In Chapter 6 an investigation was made of the effects of different protein feeding levels and different energy allowance levels during early lactation on milk production performance, on mobilisation of body tissues and on the efficiency by which ingested protein is used for

milk protein production. These feeding trials with lactating crossbred ewes involved feeding levels supplying 90%, 100% and 110% of the expected protein requirements and energy feeding levels supplied at 80% and 100% of the expected requirements. Milk production performance was not affected by protein feeding levels when the energy allowance was approximately 80% of the expected requirements, but milk production performance increased with increasing protein intake at an energy allowance of approximately 100% of the expected requirements. This was explained as plateau level production. At a constant energy allowance, milk production will increase with increasing protein intake until a plateau level is reached and this plateau level production will increase with increasing energy allowance. The calculated energy deficit during early lactation was diminished by increasing the energy allowance, whereas body weight change and body tissue mobilisation decreased. From the calculated fat and protein mobilisation it was concluded that energy supply was limiting milk production in the experiments at 80% energy allowance, whereas protein supply was limiting milk production in the experiments at 100% energy allowance. Efficiency of milk protein production was not affected by energy allowance level. This could be due to differences in mobilised energy. It was concluded that the lowest protein ingestion level and the 80% energy allowance were sufficient to maintain milk production performance.

A general discussion of the aspects of DVE evaluation for sheep is combined with the results of production and utilisation of protein for crossbred ewes. Based on the results of the experiments of Chapter 1 and 2, it is calculated that rumen passage rate is lower in sheep than in cows. It is concluded that rumen passage rate was not affected by winter shearing. DVE evaluation for sheep should take into account these differences in rumen degradation and passage kinetics, but also possible differences in efficiency of microbial protein synthesis. Literature provides efficiencies of microbial protein synthesis that are higher for sheep than for cows and are higher for winter shorn sheep than for unshorn sheep. From the results of the experiments it is concluded that crossbred ewes have higher efficiencies of microbial protein synthesis than Texel ewes. It is emphasised that the contribution of microbial protein to the DVE value is high (60-70%) and that it is important how the efficiency of microbial protein synthesis is estimated. The different DVE values that result from the above mentioned differences are combined with the experimentally derived amounts of protein needed for maintenance and production to estimate the daily DVE requirements. These requirements are in agreement with the results of the feeding trials when corrected for differences between the calculated DVE values in the experiments and DVE values from reference tables. It is concluded that the most pragmatic way to correct for differences in protein evaluation is not by correcting DVE values of feedstuffs, but by correcting DVE requirements for crossbred ewes. This approach resulted in a proposal to adapt the DVE requirements of crossbred ewes during late gestation and early lactation.

Samenvatting

Het voorliggende onderzoek richt zich op voerstrategieën voor ooien. Er zijn twee redenen om de huidige voerstrategieën te heroverwegen. Ten eerste de introductie van vruchtbare gebruikskruisingen in de schapenhouderij en ten tweede de introductie van het DVE-systeem voor de eiwitwaardering van voedermiddelen voor herkauwers.
De vruchtbare gebruikskruisingen zijn geïntroduceerd om de jaarlijkse lamsvleesproductie per ooi te verhogen. De hoge vruchtbaarheid en goede moedereigenschappen van deze kruisling ooien worden gecombineerd met de uitstekende vleesproductie kwaliteiten van de Texelaar door Texelse rammen als slachtlamvaderdier in te zetten. De voeding van deze kruisling ooien vraagt echter de aandacht, omdat de huidige voerstrategieën voor ooien bedoeld zijn voor dieren met relatief geringe worpgroottes. Het is de vraag of deze voerstrategieën ook voldoen voor vruchtbare rassen. Dit proefschrift richt zich op de eiwitbehoefte van vruchtbare ooien, omdat de energiebehoefte van vruchtbare ooien reeds onderzocht is.
Het DVE-systeem is onder andere een reactie op de toenemende maatschappelijke bezorgdheid over het effect van de (intensieve) veehouderij op het milieu. Het DVE-systeem is ontworpen voor rundvee en is een middel om het eiwitaanbod af te stemmen op de eiwitbehoefte. Dit gebeurt door middel van een gedetailleerde beschrijving van de eiwitvertering en de eiwitstofwisseling. Het DVE-systeem is gebruikt om voor verschillende voedermiddelen DVE referentiewaarden vast te stellen. Dit is gebeurd op basis van proeven met lacterende koeien. De DVE referentiewaarden worden ook gebruikt voor schapen onder de aanname dat de penskinetiek van koeien en schapen vergelijkbaar is. Er zijn echter aanwijzingen (b.v. verschillen in rantsoensamenstelling en in voerniveau) dat die aanname onjuist is.

Voor ooien zijn de late dracht en vroege lactatie twee perioden waarin het moeilijk is om de behoefte aan nutriënten te dekken uit het opgenomen rantsoen. De eiwitbehoefte van ooien bestaat in die perioden uit de hoeveelheid eiwit benodigd voor onderhoud plus de hoeveelheid eiwit benodigd voor productie van lammeren of van melkeiwit. De combinatie van de eiwitbehoefte met de efficiëntie waarmee het voereiwit wordt gebruikt voor onderhoud en productie geeft de benodigde opname van voereiwit. Ooien die drachtig zijn van een grote worp krijgen tijdens de late dracht vaak met een depressie van de voeropname te maken, hetgeen kan resulteren in een laag geboortegewicht van de lammeren. Lage geboortegewichten zijn nadelig voor het financiële resultaat van de schapenhouderij. Het geboortegewicht kan verhoogd worden door extra eiwitopname in de late dracht en door het scheren van de drachtige ooien op 6 tot 8 weken voor het aflammeren. Beide methoden worden onderzocht in dit proefschrift. De groei van de slachtlammeren is mede afhankelijk van de eiwit- (en energie)opname door de ooi gedurende het begin van de lactatie. Daarom wordt tevens de melkeiwitproductie en de efficiëntie waarmee melkeiwit wordt geproduceerd in dit proefschrift onderzocht.

De berekening van de DVE-waarde van een voedermiddel is gebaseerd op de hoeveelheid eiwit dat (per kg voedermiddel) de pens verlaat om in de dunne darm verteerd te worden.

Het eiwit dat de pens verlaat bestaat uit onafgebroken voereiwit en uit microbieel eiwit dat gevormd is in de pens. De hoeveelheid onafgebroken voereiwit wordt berekend als het complement van de hoeveelheid effectief in de pens afgebroken voereiwit. De effectieve pensafbreekbaarheid van voereiwit wordt berekend uit een combinatie van de potentieel afbreekbare eiwitfractie, de uitwasbare eiwitfractie, de afbraaksnelheid van eiwit in de pens en de passagesnelheid van eiwit door de pens. De hoeveelheid geproduceerd microbieel eiwit wordt berekend uit de combinatie van de hoeveelheid fermenteerbare organische stof (FOS) en de efficiëntie waarmee FOS wordt omgezet in microbieel eiwit. De afbraakkarakteristieken van voereiwit in de pens zijn onderhevig aan verschillende invloeden. Eén van de belangrijkste daarvan is het gevoerde rantsoen en het is alleen daarom al voorstelbaar dat koeien en schapen verschillen in afbraakkarakteristieken van voer(eiwit) in de pens. Binnen de soort schaap zijn tevens het ras en het al of niet (winter) scheren in de late dracht van grote invloed op de afbraakkarakteristieken in de pens.

Het doel van dit proefschrift was het onderzoeken van de eiwitbehoefte van ooien van een vruchtbare gebruikskruising (Flevolanders) en het valideren van het gebruik van het DVE-systeem en de huidige DVE-normen voor Flevolander ooien. Daarvoor was kennis nodig over de efficiëntie waarmee het verteerde eiwit wordt gebruikt voor onderhoud en productie en over de hoeveelheid eiwit die nodig is voor onderhoud en productie tijdens de late dracht en vroege lactatie. Voor de late dracht moest die kennis beschikbaar zijn voor zowel bewolde als geschoren ooien. Tevens moest onderzocht worden of de DVE waardering van een voedermiddel voor koeien en schapen vergelijkbaar is en of de referentiewaarden voor DVE zonder meer voor schapen gebruikt kunnen worden. Daarvoor was vergelijking van de afbraakkinetiek in de pens nodig tussen koeien en schapen, tussen Texelse en Flevolander ooien en tussen bewolde en geschoren Flevolander ooien. Ook het effect van scheren in de late dracht op de effectieve afbraak in de pens van zowel eiwit als organische stof en op de DVE waardering van voedermiddelen moest worden onderzocht. Tenslotte moesten de eiwitbehoefte en de voedernormen van Flevolander ooien tijdens de late dracht en vroege lactatie met behulp van voederproeven worden gevalideerd.

In hoofdstuk 2 worden de resultaten gepresenteerd van de gestandaardiseerde nylonzakjes techniek voor de afbraaksnelheid in de pens (Kd) en voor de niet–pensafbreekbare fractie (U) van droge stof (DS) en ruw eiwit (RE) voor koeien en schapen. Uit de resultaten bleek dat schapen meer DS en RE in de pens verteerden dan koeien. Dit verschil was het grootst tussen koeien en Texelaars, aangezien Texelaars meer nutriënten in de pens verteerden dan Flevolanders. Winter scheren tijdens de late dracht verhoogde de hoeveelheid eiwit die in de pens werd verteerd, maar de onderzochte ruwvoer/krachtvoer verhoudingen in het rantsoen hadden geen effect op de gemeten Kd en U. De uitgevoerde experimenten ondersteunen de opvatting dat de referentiewaarden voor DVE waarschijnlijk niet bruikbaar zijn voor schapen. Het lijkt er op dat bij de DVE waardering van voedermiddelen rekening moet worden gehouden met verschillen tussen koeien en schapen, tussen schapenrassen en tussen bewolde en (winter) geschoren schapen.

In hoofdstuk 3 worden de resultaten van het onderzoek naar de effectieve afbraak van voereiwit en organische stof in de pens van bewolde en (winter) geschoren Flevolander ooien beschreven. De effectieve afbraak in de pens werd geschat uit veranderingen van het pensvolume in de tijd van zowel de afgebroken als van de niet-afgebroken fracties van voereiwit en organische stof. De op deze wijze verkregen effectieve afbraak in de pens werd vergeleken met de effectieve afbraak in de pens verkregen met de standaard methode. De standaard methode combineert de *in situ* bepaalde afbraakkarakteristiek met een aangenomen passagesnelheid door de pens om de effectieve afbraak (van voereiwit) te schatten. De resultaten van de vergelijking van beide methoden werden ook gebruikt om het effect van (winter) scheren tijdens de late dracht bij Flevolander ooien te onderzoeken. Eiwitwaardering gebaseerd op veranderingen in de tijd van de pensinhoud bleek meer variatie te vertonen en berekende een 10% hogere DVE waarde dan de standaard wijze van eiwitwaardering. Het scheren van Flevolanders tijdens de late dracht verhoogde de afbraak van nutriënten in de pens. Deze verschillen resulteerden niet in verschillende hoeveelheden DVE wanneer werd aangenomen dat de efficiëntie van de microbiële eiwitsynthese niet werd beïnvloed door koude stress. Concluderend kan gesteld worden dat de standaard wijze van eiwitwaardering beter geschikt is voor routinematig onderzoek dan de methode gebaseerd op veranderingen van het pensvolume in de tijd. Daar staat tegenover dat de standaard wijze van eiwitwaardering voor Flevolanders in een onderschatting van de DVE waarde kan resulteren. Bij eiwitwaardering zou tevens rekening gehouden moeten worden met verschillen in de efficiëntie van de microbiële eiwitsynthese als gevolg van (winter) scheren om onderschatting van de DVE waarde te voorkomen.

In hoofdstuk 4 worden de resultaten van het onderzoek naar de benutting en de efficiëntie van de benutting van voereiwit in de drachtige ooi en haar foeten beschreven. Hiervoor werden stikstof (N) balansstudies met drachtige ooien uitgevoerd en werd de chemische samenstelling van pasgeboren lammeren vastgesteld. De N retentie in drachtige ooien en de N verliezen via urine werden hoger naarmate de N opname toenam. Tegelijkertijd nam de N mobilisatie uit maternaal weefsel af. Dit resulteerde in de waarneming dat de N accretie in de foeten nauwelijks werd beïnvloed door de N opname van de ooi. Uit de chemische samenstelling van de pasgeboren lammeren bleek dat bij bewolde ooien een hogere N opname in een hoger drogestof- en eiwitgehalte in de lammeren resulteerde. De over-all efficiëntie waarmee de opgenomen N werd vastgelegd in Flevolander ooien tijdens de late dracht was 66%. De onderhoudsbehoefte van deze ooien was 444 mg schijnbaar verteerde N per kg metabool lichaamsgewicht. In ooien met een (licht) positieve of een negatieve maternale N balans was de efficiëntie waarmee de opgenomen N werd vastgelegd 54% voor foetale groei en 91% voor maternale mobilisatie. Er werd geconcludeerd dat de ten behoeve van de huidige voerstrategie gebruikte deficiënties voor eiwitbenutting in de late dracht voor ooien tevens gebruikt kunnen worden voor Flevolander ooien.

In hoofdstuk 5 worden de resultaten van het onderzoek naar het effect van het eiwitaanbod en van (winter) scheren op de voeropname en de reproductiekenmerken van Flevolander

ooien tijdens de late dracht gepresenteerd. Dit werd in voederproeven onderzocht, waarbij aanbod van voereiwit werd afgestemd op een opname van ongeveer 80%, 100% en 120% van de verwachte eiwitbehoefte, waarbij het energieaanbod in overeenstemming was met de verwachte behoefte. Het effect van (winter) scheren werd onderzocht bij een beperkt en een onbeperkt aanbod van het ruwvoer (grashooi). De voeropname van de bewolde dieren veranderde nauwelijks in de late dracht en was voldoende om de verwachte energiebehoefte te dekken. De geschoren ooien lieten een verhoogde voeropname zien. De reproductie, uitgedrukt als de som van de geboortegewichten van de lammeren in één worp, werd niet beïnvloed door verschillen in eiwitopname. Dit duidt erop dat de drie aangeboden eiwitniveaus allemaal voldoende waren om de eiwitbehoefte van de ooien te dekken. De geschoren ooien wierpen gemiddeld zwaardere lammeren, ondanks dat de eiwitopname vergelijkbaar was met dat van de bewolde dieren op het hoogste eiwitopname niveau. Hiervoor werden drie mogelijke verklaringen gegeven: de reproductie kan verbeterd worden door het aanbod van zowel eiwit als energie te verhogen, het eiwitaanbod van de geschoren dieren werd mogelijk onderschat en (winter) scheren induceert een andere verdeling van de beschikbare nutriënten tussen moeder en foeten. De behandelingen hadden geen invloed op de verandering in conditie score van de ooien of op de wolgroei, maar geschoren ooien verloren meer lichaamsgewicht gedurende de late dracht. Dit werd toegeschreven aan een hogere depositie in de lammeren en een hogere onderhoudsbehoefte voor energie van de ooien. Er werd geconcludeerd dat het mogelijk is de voeropname tijdens de late dracht constant te houden door een rantsoen met een toenemende verhouding krachtvoer/ruwvoer te voeren, dat de huidige voedernormen voor eiwit tijdens de late dracht verlaagd kunnen worden voor Flevolander ooien en dat (winter) scheren tijdens de late dracht het gemiddelde geboortegewicht van de lammeren verhoogd.

In hoofdstuk 6 worden de resultaten van het onderzoek naar het effect van eiwitopname niveau in combinatie met energieopname niveau op de melkproductie, de mobilisatie van lichaamsreserves en op de efficiëntie waarmee het opgenomen eiwit gebruikt wordt voor de melkeiwitproductie beschreven voor Flevolander ooien tijdens de vroege lactatie. In voederproeven gedurende de eerste 6 weken van de lactatie werden 3 eiwitopname niveaus gecombineerd met 2 energieopname niveaus. De voerniveaus waren voor eiwit ongeveer 90%, 100% en 110% van de verwachte eiwitbehoefte en voor energie ongeveer 80% en 100% van de verwachte energiebehoefte. Bij een energieaanbod van ongeveer 80% van de verwachte behoefte werd de hoeveelheid geproduceerde melk niet beïnvloed door het eiwitaanbod, maar bij een energieaanbod van ongeveer 100% van de verwachte behoefte resulteerde extra eiwitopname in een verhoogde melkproductie. Dit verschil werd verklaard met de theorie van productieplateaus voor melkproductie. Bij een gelijke energieopname zal de melkproductie stijgen met een stijgende eiwitopname totdat het productieplateau is bereikt. Dit productieplateau is gerelateerd aan de energieopname en stijgt bij een stijgende energieopname. Het berekende energietekort in de vroege lactatie kon worden verkleind door een hoger energieaanbod, wat tevens resulteerde in een verminderde mobilisatie van lichaamsreserves. Uit de berekende mobilisatie van vet en eiwit werd geconcludeerd dat bij

een energieaanbod van ca. 80% van de behoefte de energieopname de beperkende factor voor melkproductie was, terwijl bij een energieaanbod van ca. 100% van de behoefte juist de eiwitopname de beperkende factor voor melkproductie was. De efficiëntie waarmee het opgenomen eiwit werd gebruikt voor melkeiwitproductie werd niet beïnvloed door het energieaanbod. Dit kan mogelijk verklaard worden uit de verschillen in de gemobiliseerde energie. Er werd geconcludeerd dat het laagste eiwitopname niveau (9 g DVE/kg metabool gewicht) en een energieaanbod van ongeveer 80% van de behoefte voldoende waren voor een goede melk(eiwit)productie.

In de algemene discussie worden de verschillende aspecten van de DVE waardering voor schapen gecombineerd met de in de voederproeven geconstateerde eiwitproductie en eiwitbenutting van Flevolander ooien. Gebaseerd op de resultaten van de hoofdstukken 1 en 2 werd berekend dat de passagesnelheid door de pens van schapen lager is dan die van koeien. Ook werd geconcludeerd dat de passagesnelheid door de pens van Flevolanders nauwelijks werd beïnvloed door (winter) scheren tijdens de late dracht. De DVE waardering van voedermiddelen zou voor schapen niet alleen rekening moeten houden met de geobserveerde verschillen in pensafbraaksnelheid en penspassagesnelheid tussen koeien en schapen, maar ook met mogelijke verschillen in de efficiëntie waarmee FOS wordt omgezet in microbieel eiwit. Literatuur geeft aan dat de efficiëntie van de microbiële eiwitsynthese hoger is in schapen dan in koeien en ook hoger in schapen met (geschoren) of zonder (bewold) koude stress. De resultaten van de experimenten geven aan dat de efficiëntie van de microbiële eiwitsynthese in Flevolander ooien hoger is dan in Texelse ooien. Er werd benadrukt dat de bijdrage van microbieel eiwit aan DVE hoog is (60-70%) en dat het daarom belangrijk is dat de efficiëntie van de microbiële eiwitsynthese goed wordt ingeschat. De bovengenoemde verschillen resulteerden tevens in verschillende DVE waarderingen voor het rantsoen. Deze verschillende DVE waarden werden gecombineerd met de experimenteel vastgestelde hoeveelheden voereiwit die nodig zijn voor productie en onderhoud om de dagelijkse DVE behoefte van Flevolander ooien tijdens de late dracht en vroege lactatie te kunnen schatten. De op deze wijze geschatte DVE behoeftes kwamen goed overeen met de resultaten van de voederproeven wanneer gecorrigeerd werd voor verschillen tussen de in de experimenten berekende DVE waarde en de standaard DVE waarde. Er werd geconcludeerd dat de meest pragmatische manier om deze verschillen te corrigeren niet ligt bij het aanpassen van de DVE waarde van de voedermiddelen, maar bij het aanpassen van de DVE normen voor Flevolander ooien. Deze aanpak resulteerde dan ook in een voorstel om de DVE normen voor Flevolander ooien tijdens de late dracht en vroege lactatie aan te passen.

Curriculum Vitae

Léonhard Bohumil Johannes Šebek werd geboren op 28 oktober 1958 te Apeldoorn. In 1978 behaalde hij het Atheneum-B diploma aan het Marianum te Groenlo en begon hij aan de studie Biologie aan de Universiteit van Utrecht. In 1979 stopte hij met die studie en ging in juli met een vervroegde aanvraag militaire dienst onder de wapenen om vervolgens eind oktober 1980 als Luitenant Logistiek af te zwaaien. Daarna volgde een periode van 8 maanden waarin hij via een uitzendbureau als productiemedewerker bij de vestigingen van achtereenvolgend Grolsch, Vredestein en Polaroid te Enschede werkte. Vervolgens werd gestudeerd aan de RHLS te Groningen waar hij in 1985 het diploma behaalde van de afstudeerrichting Veehouderij met als specialisatie veevoeding en fokkerij. In september 1985 trad hij in tijdelijke dienst als onderzoeksassistent bij het Instituut voor Veevoedingsonderzoek te Lelystad. Het werkterrein betrof voederwaarde onderzoek bij varkens. Na ruim 4 jaar volgde een aanstelling in vaste dienst en werd het werkterrein verlegd naar voedingsonderzoek bij schapen. Wederom na ruim 4 jaar werd het werkterrein verlegd naar voedingsonderzoek bij herkauwers in het algemeen en bij melkkoeien in het bijzonder. In juni 2001 werd van werkgever gewisseld en sindsdien werkt hij als wetenschappelijk onderzoeker op het terrein van de voeding van herkauwers bij het Praktijkonderzoek Veehouderij te Lelystad.