Supplementation of lactating Dorper and Merino ewes on *Themeda cymbopogon* veld. 3. Seasonal and diurnal variation in rumen pH and ammonia concentration

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Received 14 April 1988; accepted 8 August 1989

Extract from Ph.D (Agric.) thesis submitted by HOdeW to the Department of Animal Science, University of Stellenbosch, 1986.

The effects of energy and crude protein (CP) supplementation on diurnal and seasonal variations in rumen pH and concentrations of ammonia (NH_3) in lactating ewes grazing native pasture (veld) were studied during two autumn (1981 and 1982) and two spring (1983 and 1984) lambing seasons. Different levels of energy and / or CP were provided daily via rumen fistulae to the ewes. Considerable diurnal and seasonal variation in rumen pH was observed between and within the winter (1981 and 1982) and summer (1983 and 1984) seasons. This was ascribed to seasonal differences in grazing pattern, herbage intake and ruminal activity of grazing sheep. In summer, ruminal pH was lower and increased between 08h00 and 12h00, while in winter it decreased during the same period. Prevailing ruminal pH of Dorper ewes was always lower than for Merino ewes. Supplementary CP and CP plus energy influenced ruminal pH. The small effect of supplementary energy on ruminal pH indicated that much of the maize starch was apparently digested postruminally. Concentration of rumen NH_3 was higher in summer, but with no definite pattern of diurnal variation discernible within seasons. Prevailing concentrations of ruminal NH_3 of Dorper ewes were lower during winter than those of Merinos. During winter (1981 and 1982), concentration of rumen NH_3 was markedly increased by supplementary CP but not affected by energy, while CP plus energy resulted in intermediate NH_3 levels. In summer (1983), rumen NH_3 concentration was increased by CP and decreased by energy, while CP plus energy resulted in intermediate rumen NH_3 levels. During the second trial in summer (1984), supplementary energy did not appear to affect rumen NH_3 concentration. Diurnal and seasonal variation in ruminal pH and NH_3 levels are discussed in relation to feed intake and animal performance.

Die invloed van aanvullende energie en ruproteïen (RP) op die daaglikse en seisoenale variasie in rumen-pH en ammoniak(NH_3)-konsentrasie van lakterende ooie op veldweiding, is gedurende twee herfs- (1981 en 1982) en twee lentelamseisoene (1983 en 1984) ondersoek. Verskillende peile energie en / of RP is daagliks via rumenfistels aan die ooie verskaf. Aansienlike daaglikse en seisoenale variasie in rumen-pH is tussen en binne die winter (1981 en 1982) en somer (1983 en 1984) waargeneem. Dit is aan seisoenale verskille in weipatroon, inname en rumenaktiwiteit van weidende skape toegeskryf. Rumen-pH was laer gedurende die somer en het vanaf 08h00 tot 12h00 gestyg en in die winter vanaf 08h00 na 12h00 gedaal. Rumen-pH van Dorperooie was deurgaans laer as dié van Merino-ooie. Aanvullende RP, sowel as RP plus energie, het rumen-pH beïnvloed. Die geringe invloed van aanvullende energie op rumen-pH dui daarop dat heelwat mieliestysel waarskynlik postruminaal verteer is. Rumen-NH_3 was hoër gedurende die somer, maar geen definitiewe daaglikse patroon van variasie is binne seisoene waargeneem nie. Rumen-NH_3 van Dorperooie was laer as die van Merino-ooie gedurende die winter. In die winter (1981 en 1982) is NH_3 aansienlik deur aanvullende RP verhoog, terwyl energie geen effek gehad het nie en RP plus energie intermediêre NH_3-vlakke gelewer het. In die somer (1983) is NH_3 verhoog deur RP en verlaag deur energie, terwyl RP plus energie ook intermediêre vlakke gelewer het. In die tweede somer (1984) het aanvullende energie weinig invloed op NH_3 uitgeoefen. Daaglikse en seisoenale variasie in rumen-pH en -NH_3 word bespreek in verhouding tot voedingstofinname en dierepresentasie.

Keywords: Crude protein, energy, native pasture (veld), reproducing sheep, rumen NH_3, rumen pH, supplementation

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Introduction

By creating more optimum conditions in the rumen for the micro-organisms, supplements, stimulating the rumen, may increase both feed intake and digestibility of low-quality roughages (Van Niekerk & Van der Merwe, 1966; Swart, Niemann, Engels & Biel, 1971; Louw, 1978). Yet, in recent studies on native pasture (veld) with Merino and Dorper wethers (De Waal, Engels, Van der Merwe & Biel, 1981), young Merino wethers (De Waal, Baard & Engels, 1989b) and lactating Merino and Dorper ewes (De Waal & Biel, 1989b), supplementary crude protein (CP) did not significantly affect the herbage intake. Apparently, rumen activity as influenced by the herbage ingested was not suboptimal and could therefore not have been influenced by supplementary CP. Alternatively, the selective grazing behaviour of sheep being a time consuming process, simply prohibited any response, as suggested (De Waal & Biel, 1989b). Furthermore, a substitution effect on herbage intake by the ewes in response to supplementary energy was present, the substitution effect being greater during summer (De Waal & Biel, 1989b).

Utilization of potential energy sources such as starch, cellulose and hemicellulose by ruminants and the quantity and quality of amino acids available for absorption in the lower alimentary tract, depends on the

activity of the micro-organisms in the rumen (Chalupa, 1975; Ørskov, 1975; Satter & Roffler, 1975; Ørskov, 1977; Chalupa, 1984). Metabolic activity in the rumen usually reaches a peak within a few hours after feeding (Blackburn, 1965; Warner, 1965), with a concomitant decline in ruminal pH (Du Plessis & Van der Merwe, 1970; Leng, 1970; Pritchard & Males, 1982). Diurnal variation in rumen pH is therefore an indication of the accumulation of organic acids in the rumen as a result of fermentation (Leng, 1970), as well as the excretion of saliva into the rumen (Church, 1973). Ammonia (NH_3) is utilized by most rumen bacteria as a primary nitrogen (N) source (Bryant & Robinson, 1963; Hogan, 1975; Satter & Roffler, 1975; Chalupa, 1975; Schaefer, Davis & Bryant, 1980; Chalupa, 1984), but maximum rumen microbial protein synthesis depends on the availability of NH_3 in the presence of suitable fermentable substrates (Weston & Hogan, 1968; Hogan & Weston, 1970; Hogan, 1975; Satter & Roffler, 1975; Ørskov, 1977; Stern & Hoover, 1979; Chalupa, 1984). In this regard, 2—5 mg NH_3-N per 100 ml rumen fluid has been suggested as the optimum concentration for microbial protein synthesis (Satter & Slyter, 1974; Hogan, 1975; Satter & Roffler, 1975). Rumen pH plays a major role in the absorption of both NH_3 (Bloomfield, Kearley, Creach & Muhrer, 1963; Blackburn, 1965; Leng & Nolan, 1984) and volatile fatty acids (VFA) from the rumen (Bloomfield *et al.*, 1963; Macleod, Ørskov & Atkinson, 1984). Ionization of ammonia (NH_3) to ammonium (NH_4fa), is suppressed by a high pH, thus more NH_4fa is present at a lower pH. Ammonia is absorbed more rapidly in the un-ionized state (NH_3) (Bloomfield *et al.*, 1963; Blackburn, 1965; Leng & Nolan, 1984), therefore NH_3-N is retained longer in the rumen at a lower ruminal pH and, in the presence of suitable fermentable substrates, may result in higher microbial protein yields (Blackburn, 1965; Leng, 1984).

Rumen pH and NH_3 levels may therefore provide valuable information on diurnal and seasonal changes in the activity of ruminal micro-organisms, as well as short-term responses to supplements. Since there is a paucity of information on these rumen parameters for free-ranging ruminants and the way in which they are influenced by supplementary feeding (De Waal, Engels & Van der Merwe, 1980; De Waal *et al.*, 1989b), it was decided to conduct this study.

Procedure

The experimental site, animals used, general procedures and supplementation levels have been described by De Waal & Biel (1989a; 1989b). Supplements were provided daily via rumen fistulae to lactating ewes in 1981 and 1982 (autumn lambing seasons) and 1983 and 1984 (spring lambing seasons).

In all trials, rumen fluid was sampled on four consecutive days from ewes which were on average three weeks post partum. To obtain an indication of the diurnal variation in rumen pH and NH_3 concentrations, the following sampling schedule, as suggested by De Waal *et al.* (1989b), was used:

Day 1 sample at 08h00
Day 2 sample at 12h00
Day 3 sample at 16h00
Day 4 sample at 20h00

Rumen fluid (*ca.* 300 ml) was aspirated via the rumen fistula by means of a stomach tube connected to a flexible plastic container (1 *l* capacity). The pH of the rumen fluid was measured immediately with a portable digital pH meter and the fluid strained through four layers of cheese cloth. A subsample of 100 ml strained rumen fluid was acidified with 1 ml of concentrated H_2SO_4 in a small plastic bottle with a screw cap and stored at –15°C. Prior to analysis, samples were thawed and NH_3 concentrations were determined by distillation over MgO as described by De Waal (1986) and expressed as mg NH_3 per 100 ml rumen fluid.

Results

Rumen pH and concentrations of NH_3 in the rumen fluid of ewes in the respective trials and treatment groups are presented in Figures 1 to 12. The mean effects of the various supplements on the rumen parameters are presented in Tables 1 and 2.

In 1981 (autumn / winter), ruminal pH decreased between 08h00 and 20h00 for both Merino and Dorper ewes (Figures 1 and 2). In 1982, ruminal pH decreased for both breeds between 08h00 and 16h00, whereafter pH increased (Figures 3 and 4). The ruminal pH of both breeds was also lower in 1981 than in 1982 (Figures 1 and 2 *vs.* 3 and 4). In contrast, in 1983 (spring/summer) ruminal pH of the Dorper ewes increased markedly between 08h00 and 12h00 (Figure 5). From 12h00 to 16h00 relatively little variation was observed in ruminal pH and between 16h00 and 20h00 pH decreased again.

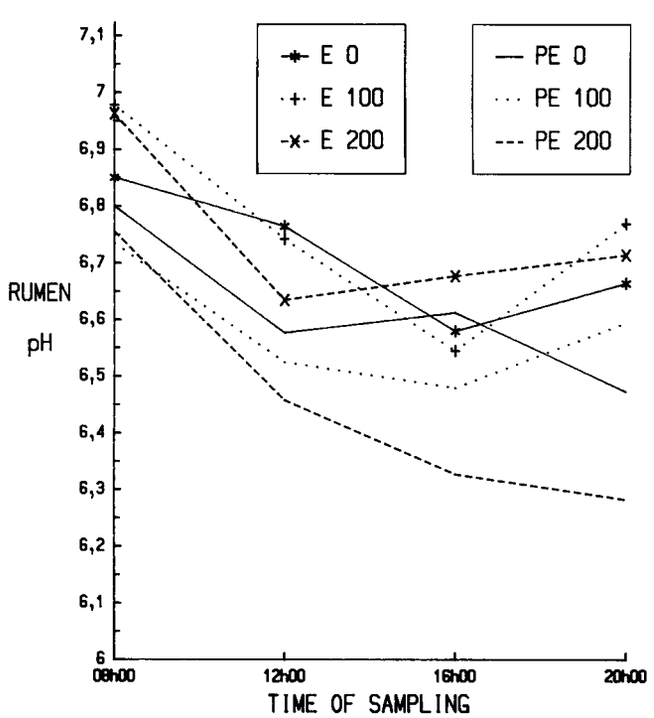

Figure 1 The rumen pH of lactating Merino ewes at fixed intervals after supplementation at 08h00 in 1981

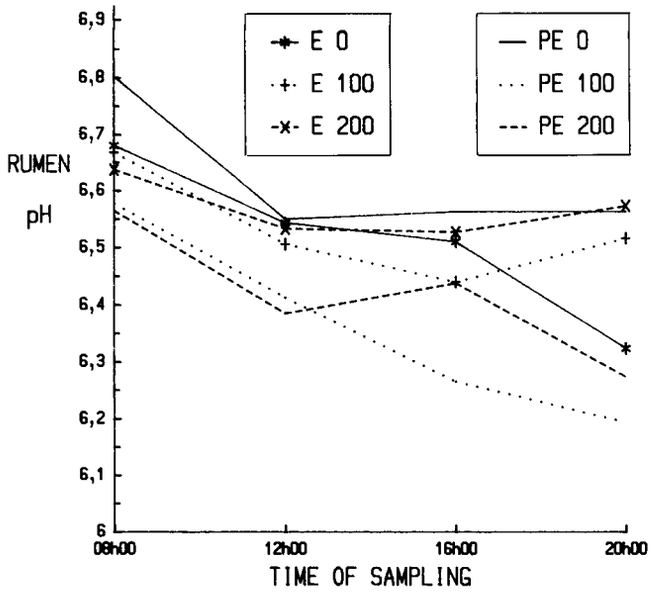

Figure 2 The rumen pH of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1981

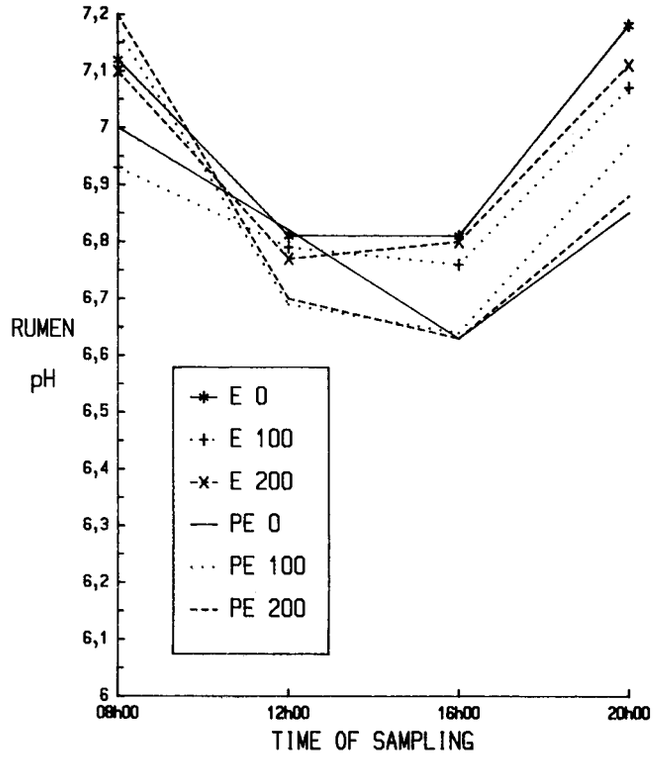

Figure 3 The rumen pH of lactating Merino ewes at fixed intervals after supplementation at 08h00 in 1982

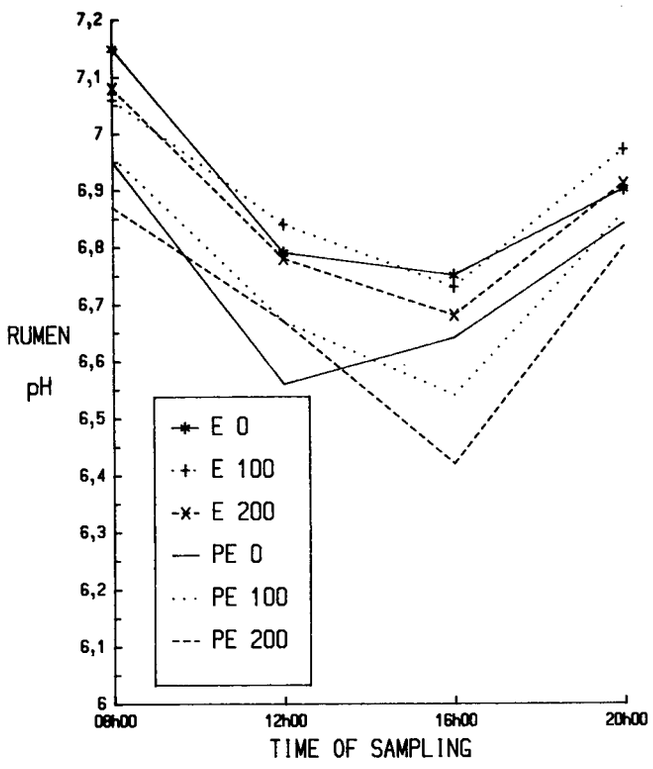

Figure 4 The rumen pH of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1982

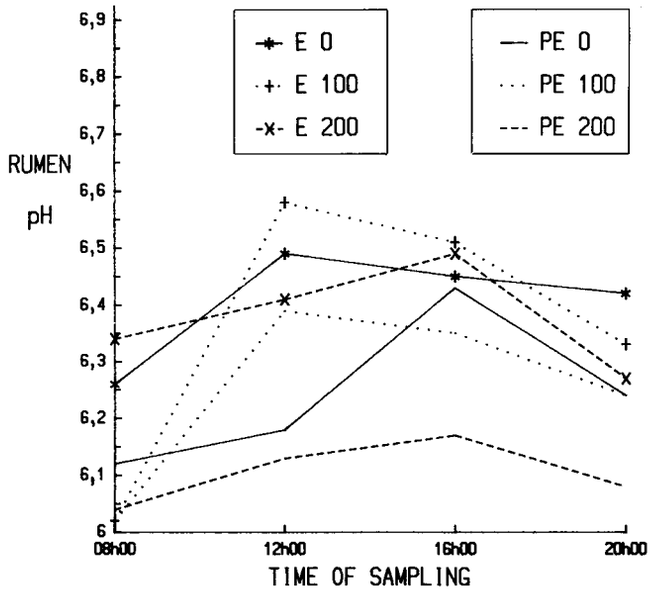

Figure 5 The rumen pH of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1983

During 1984 (Figure 6), ruminal pH of the Dorper ewes also increased between 08h00 and 12h00, whereafter it decreased between 12h00 and 16h00 and then increased till 20h00.

Supplementary energy (E 100 and E 200) did not affect ruminal pH of either breed in 1981 (Figures 1 and 2). Supplementary CP, with the highest level of energy (PE 200), decreased ruminal pH of the Merino ewes (Figure 1). Supplementary energy (E 100 and E 200) also did not affect ruminal pH of either breed in 1982, while supplementary CP, with or without energy (PE 0, PE 100 and PE 200), decreased ruminal pH of both breeds (Figures 3 and 4). The observations with Dorpers in 1983 (Figure 5) were similar. In 1984 (Figure 6), the respective treatments (E 150, E 300 and E 450) had little effect on ruminal pH of Dorper ewes.

Supplementary energy (E 100 and E 200) did not appear to affect concentrations of ruminal NH_3 of either breed during the trials in winter (1981 and 1982; Figures 7 to 10). However, in summer (1983) supplementary

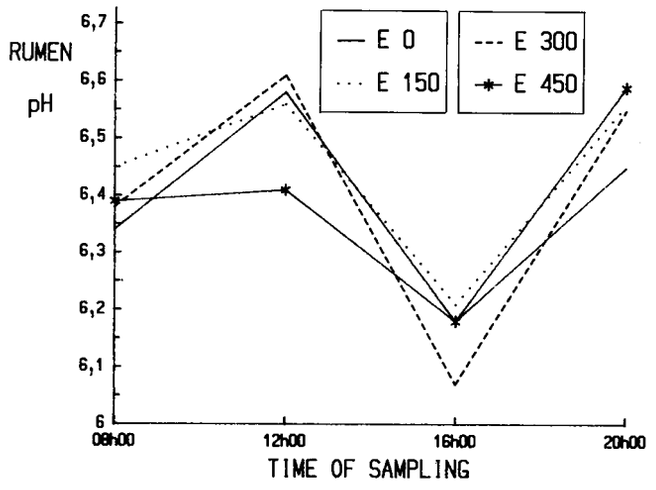

Figure 6 The rumen pH of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1984

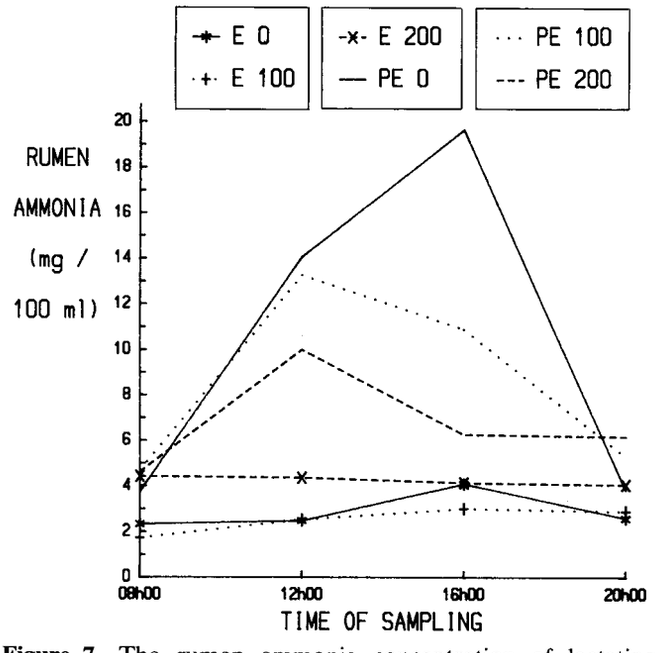

Figure 7 The rumen ammonia concentration of lactating Merino ewes at fixed intervals after supplementation at 08h00 in 1981

energy (E 100 and E 200) tended to suppress ruminal NH_3 concentrations in Dorper ewes (Figure 11). In the next summer (1984; Figure 12), supplementary energy (E 150, E 300 and E 450) caused reduced ruminal NH_3 concentrations only at midday (12h00). Supplementary CP (PE 0) increased rumen NH_3 concentrations substantially in all trials (Figures 7 to 11), the highest levels usually being observed at 12h00, i.e. 4 h after supplementation. However, in some cases (Figures 7 and 8), the highest concentrations were only observed at 16h00. Having reached peak values, ruminal NH_3 concentrations declined sharply, where the levels reached at 20h00 were again comparable to those observed at 08h00. Provision of supplementary CP plus energy (PE 100 and PE 200), resulted in smaller peak ruminal NH_3 concentrations in comparison to supplementary CP (PE 0) only (Figures 7 to 12).

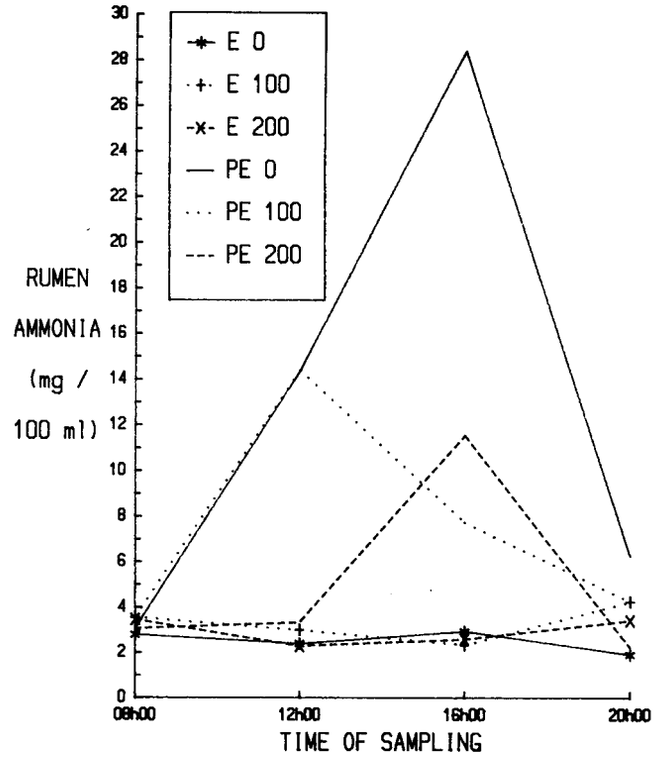

Figure 8 The rumen ammonia concentration of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1981

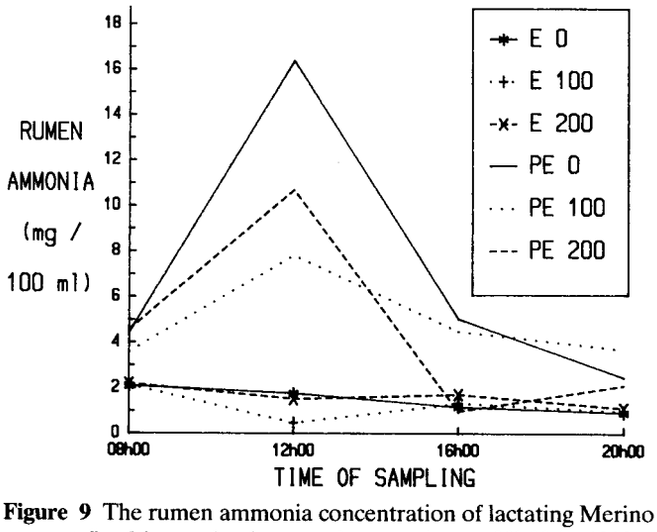

Figure 9 The rumen ammonia concentration of lactating Merino ewes at fixed intervals after supplementation at 08h00 in 1982

Discussion

In this study, 4, 8, 12 and 24 h had elapsed respectively during Days 2, 3, 4 and 1 between supplementation at 08h00 and sampling of rumen fluid. Although the samples were taken on four different days, the mean interval between sampling times was still regarded as being 4 h. An interval of 4 h between samplings is obviously too long to detect transient shifts in rumen parameters. Therefore, peak or low values for rumen pH and NH_3, as depicted in the respective figures, do not necessarily reflect the absolute highest or lowest values for a particular parameter, but merely reflect the values at the time of sampling.

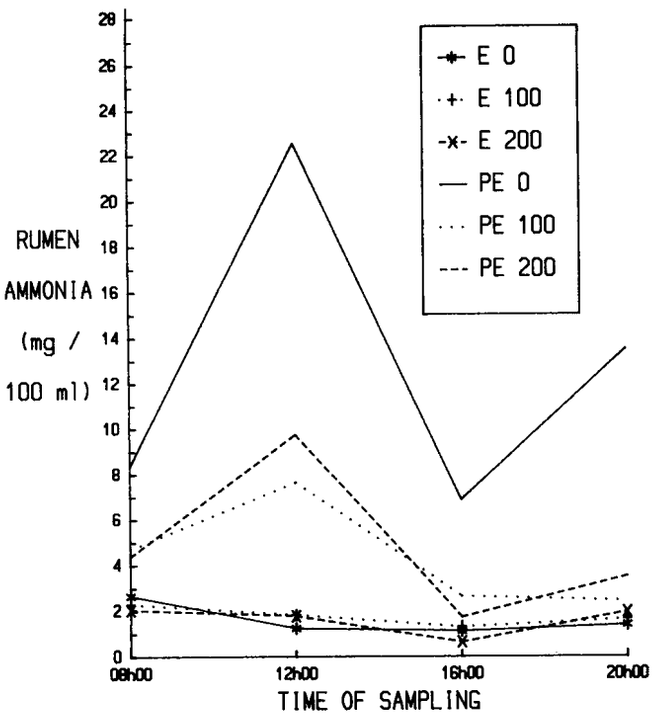

Figure 10 The rumen ammonia concentration of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1982

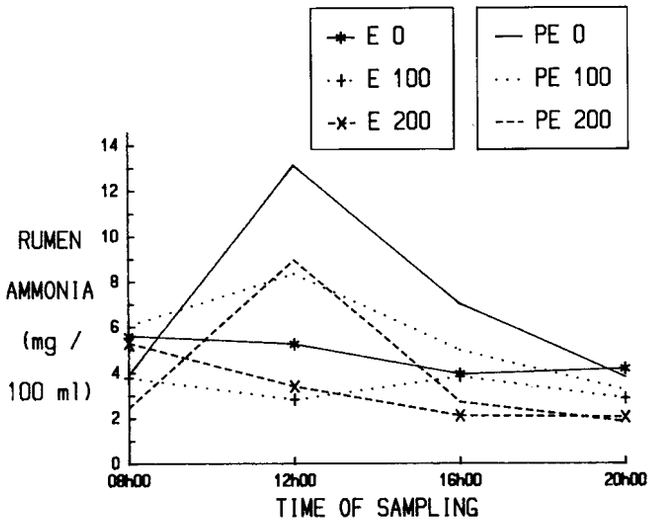

Figure 11 The rumen ammonia concentration of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1983

Considerable diurnal as well as seasonal variation in ruminal pH was observed. The largest differences in seasonal and diurnal pH variation were observed between winter (1981 and 1982) and summer (1983 and 1984). Moreover, ruminal pH also varied between the two winter trials (1981 *vs.* 1982), as well as the two summer trials (1983 *vs.* 1984). The decrease in rumen pH between 08h00 and 20h00 (Figures 1 and 2), or between 08h00 and 16h00 (Figures 3 and 4), as well as the increase in pH between 08h00 and 12h00 (Figures 5 and 6), may be attributed to the grazing habits and pattern of herbage intake by the sheep in autumn /

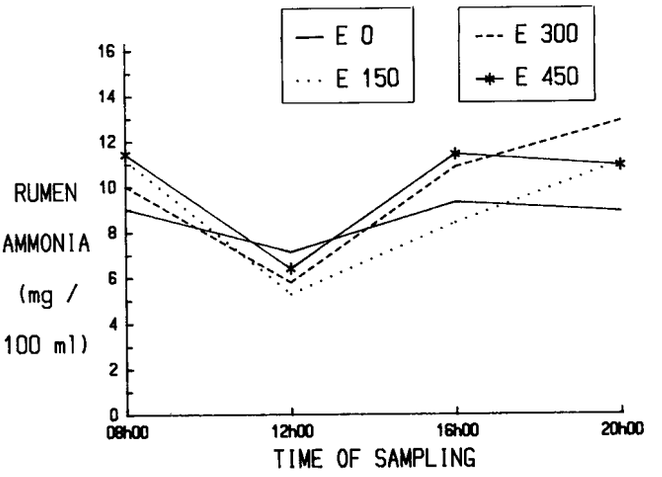

Figure 12 The rumen ammonia concentration of lactating Dorper ewes at fixed intervals after supplementation at 08h00 in 1984

Table 1 The effect of energy and crude protein supplementation on the average rumen pH of lactating Merino and Dorper ewes on veld

Trial period	Breed	Treatment					
		E 0	E 100	E 200	PE 0	PE 100	PE 200
1981	Merino	6,72[a]	6,70[a]	6,75[a]	6,62[a]	6,59[a]	6,46[a]
	Dorper	6,51[a]	6,53[a]	6,57[a]	6,62[a]	6,43[a]	6,42[a]
1982	Merino	6,98[a]	6,89[a]	6,95[a]	6,83[a]	6,87[a]	6,85[a]
	Dorper	6,90[a]	6,90[a]	6,86[ac]	6,75[bc]	6,76[bc]	6,69[b]
1983	Dorper	6,40[a]	6,36[a]	6,38[a]	6,24[c]	6,25[c]	6,10[b]

		Treatment			
		E 0	E 150	E 300	E 450
1984	Dorper	6,39[a]	6,45[a]	6,41[a]	6,36[a]

[a,b,c] Within trial periods and breeds, averages with the same superscript do not differ significantly ($P \leq 0,05$); *t* test (Harvey, 1976).

Table 2 The effect of energy and crude protein supplementation on the average rumen ammonia concentration (mg NH_3 / 100 ml) of lactating Merino and Dorper ewes on veld

Trial period	Breed	Treatment					
		E 0	E 100	E 200	PE 0	PE 100	PE 200
1981	Merino	2,87[ac]	2,55[a]	4,25[ac]	10,32[b]	8,54[b]	6,76[bc]
	Dorper	2,51[a]	3,30[a]	2,95[a]	13,03[c]	7,50[a]	5,05[a]
1982	Merino	1,49[a]	1,26[a]	1,64[a]	7,08[b]	4,90[b]	4,59[b]
	Dorper	1,61[a]	1,78[a]	1,61[a]	13,62[b]	4,46[a]	4,85[a]
1983	Dorper	4,73[ac]	3,33[ac]	3,21[ac]	6,96[b]	5,66[bc]	3,99[c]

		Treatment			
		E 0	E 150	E 300	E 450
1984	Dorper	8,59[a]	8,99[a]	9,89[a]	10,04[a]

[a,b,c] Within trial periods and breeds, averages with the same superscript do not differ significantly ($P \leq 0,05$); *t* test (Harvey, 1976).

winter and spring / summer respectively (De Waal, 1986). Diurnal variation in concentration of organic acids in the rumen and thus ruminal pH, is primarily determined by the feeding schedule and not the time of day at which it is measured (Warner, 1965). In conventional studies with pen-fed ruminants, rumen fluid is usually sampled at fixed intervals after feeding (Du Plessis & Van der Merwe, 1970). In this study and the studies of De Waal *et al.* (1980) and De Waal *et al.* (1989b), rumen fluid was sampled at fixed hours of the day, with the supplements administered via the rumen cannulae at 08h00 to the grazing sheep. Although the feed intake of grazing sheep may be regarded as a continuous process, there are certain times of the day when they are not grazing. Previous studies showed that sheep start actively grazing at sunrise (05h30) in summer and wait for sunrise in winter (07h00) (De Waal *et al.*, 1980; De Waal *et al.*, 1989b). The present study confirmed these observations. Since rumen fluid was initially sampled at 08h00, the sheep had an opportunity to graze before sampling commenced. Therefore, samples taken at 08h00 in winter (Figures 1 to 4) could be regarded as representative of a phase during or just after feeding (Walker & Nader, 1970; De Waal *et al.*, 1980), whereas the samples taken at 08h00 in summer (Figures 5 and 6) represented a longer post-feeding phase. The ruminal metabolic activity usually reaches a peak within a few hours after feeding (Warner, 1965), with a concomitant decline in ruminal pH (Du Plessis & Van der Merwe, 1970; Leng, 1970). The decline in ruminal pH between 08h00 and 20h00 (Figures 1 and 4) or 08h00 and 16h00 (Figures 3 and 4) may, therefore, be ascribed to increased metabolic activity in the rumen. Conversely, the increase in ruminal pH between 08h00 and 12h00 (Figures 5 and 6) results from a progressive post-feeding decrease in ruminal metabolic activity. These observations suggest that in summer, with high day temperatures, sheep prefer to graze actively during the cooler pre- and post-dawn and the pre- and post-dusk periods of the day. In winter, however, active grazing occurs mainly during the period of daylight.

Figures 1 to 4 and Table 1 indicate that lower pH values are found in Dorper ewes than Merino ewes. Lower ruminal pH values are indicative of higher ruminal activity (Leng, 1970). Corresponding differences in ruminal pH for non-lactating Merino and Dorper ewes were reported by De Waal (1986). This difference therefore occurs during physiological states other than lactation. Olson, Cramer & Nagy (1968) found that breeds could differ with respect to ruminal pH. Their results should, however, be viewed with caution, as ruminal pH was only determined 30 minutes after slaughtering, prior to which the sheep were withheld from food and water for 16 h. In the present study the sheep were not fasted or withheld from water. The ruminal pH is therefore a reflection of the rumen activity of free-ranging sheep.

The sharp drop in ruminal pH after 16h00 in some treatments may have affected the cellulolytic bacteria (Terry, Tilley & Outen, 1969; Mann & Ørskov, 1975; Mackie, Gilchrist, Robberts & Schwartz, 1978), which would have led to a concomitant reduction in digestion of cellulose and hemicellulose (Terry *et al.*, 1969; Armstrong & Smithard, 1979; Henning, Van der Linden, Mattheyse, Nauhaus, Schwartz & Gilchrist, 1980; Ørskov, 1982). The low ruminal pH of ewes in these treatments may be ascribed to sustained elevation of VFA concentrations (De Waal *et al.*, 1989b). According to Ørskov (1982), the rate of cellulose digestion falls rapidly to zero at rumen pH of $< 6,1$ to 6,2, but the extent to which cellulose digestion is depressed depends both on the duration and magnitude of the prevailing decrease in ruminal pH. In contrast, De Waal *et al.* (1989a; 1989b) found that, although the ruminal pH of grazing Merino wethers varied between 6,48 (08h00) and 6,12 (20h00) in winter, their performance was apparently not affected. Furthermore, in 1983 (Figure 5), the rumen pH of Dorper ewes in Treatment PE 200 also varied between 6,04 (08h00) and 6,17 (20h00), yet they performed exceptionally well (De Waal & Biel, 1989a). Since ruminal pH was only measured over a '12 h period' (08h00 to 20h00), it is not possible to predict pH changes between 20h00 and 08h00. It can be assumed, however, that by morning the ruminal pH had returned to the values observed at 08h00, thus completing the diurnal cycle. The higher prevailing ruminal pH in 1982 may have affected the absorption of NH_3 (Bloomfield *et al.*, 1963; Blackburn, 1965; Leng & Nolan, 1984), as well as that of VFAs (Bloomfield *et al.*, 1963; Macleod *et al.*, 1984). This accounted, to some extent, for the general decrease in animal performance during 1982 (De Waal & Biel, 1989a).

The observed decrease in rumen pH, in response to supplementary CP and energy (PE 0, PE 100 and PE 200), apparently resulted from an increase in ruminal activity. Contrary to published results (El–Shazly, Dehority & Johnson, 1961; Reed, Elliott & Topps, 1968; Terry *et al.*, 1969; Du Plessis & Van der Merwe, 1970; Mackie *et al.*, 1978; Henning *et al.*, 1980; Hynd, 1984), ruminal pH was not observed to be affected by supplementary energy (E 100 and E 200). This may be attributed to:

(i) the small contribution made by the supplements (even at 450 g maize day^{-1}) in relation to the daily herbage intake by ewes (De Waal & Biel, 1989b);
(ii) differences between grazing and pen-fed sheep. In a study by Du Plessis & Van der Merwe (1970), 250 g maize day^{-1} significantly affected ruminal pH of sheep kept in pens when supplementing freshly cut lucerne, yet little response in rumen pH was discernible when the sheep were allowed to graze lucerne pasture. The authors ascribed this to differences in feed intake, selective grazing behaviour and the period over which the feed was ingested;
(iii) retention time of maize in the rumen, owing to the small particle size (Tucker, Mitchell & Little, 1968; Ørskov, Fraser & Kay, 1969; Mehrez & Ørskov, 1978; Ørskov, 1982), may have prevented extensive fermentation. Furthermore, uncooked maize starch

is relatively resistant to ruminal fermentation (Tucker *et al.*, 1968; Karr, Little & Mitchell, 1966; Ørskov *et al.*, 1969). Ørskov *et al.* (1969) and Tucker *et al.* (1968) showed that 14—25% and 38% maize starch, respectively, escaped rumen fermentation and was digested postruminally.

De Waal *et al.* (1980) suggested that, owing to the more continuous process of feed intake by grazing ruminants, a small diurnal variation in ruminal NH_3 is to be expected. The results of this study and the studies of De Waal (1986) and De Waal *et al.* (1989b) are in general agreement with this suggestion. Moreover, the distinct diurnal variation in rumen pH, within seasons, did not correspond to rumen NH_3 concentrations (Figures 7 to 10). The winter ruminal NH_3 of the lactating Merino and Dorper ewes in the Control groups (E 0) varied between a narrow margin of 1—3 mg NH_3 / 100 ml rumen fluid. During summer, however, the ruminal NH_3 of Dorper ewes in the Control groups (E 0) was mostly in excess of 3 mg (Figure 11), often to as high as 7 mg NH_3 / 100 ml rumen fluid (Figure 12). These differences in prevailing ruminal NH_3 levels can be attributed mainly to differences in CP content and also, to a lesser extent, the organic matter digestibility (OMD) of the herbage ingested by the ewes (De Waal & Biel, 1989b). Assuming that 2—5 mg NH_3-N / 100 ml rumen fluid is the optimum concentration for microbial protein synthesis (Hogan, 1975; Satter & Roffler, 1975) and allowing for differences in expressing rumen NH_3 concentration (NH_3 *vs.* NH_3-N), some of the rumen NH_3 levels could well have been suboptimal for bacterial protein synthesis in winter. Supplementary CP (PE 0) invariably resulted in markedly elevated rumen NH_3 levels, with Dorper ewes reaching the highest peak ruminal NH_3 concentrations (Figures 7 to 10). The elevated NH_3 levels, in response to supplementary CP, resulted from the rapid hydrolysis of urea to NH_3 upon entering the rumen (Drori & Loosli, 1961; Coetzee, Lesch & Nel, 1967; Coetzee & Lesch, 1969; Chalupa, Clark, Opliger & Lavker, 1970; Leng & Nolan, 1984). The sustained high level of NH_3 may be accounted for by recycling of N via the saliva and, to a lesser extent, through the rumen wall (Martin & Blaxter, 1965; Weston & Hogan, 1968; Hogan, 1975; Kennedy, 1980; Obara & Shimbayashi, 1980; Leng & Nolan, 1984). The sustained high levels of NH_3 in some treatments (PE 0, PE 100 and PE 200), especially during winter (1981 and 1982), may be indicative of a relatively inactive rumen population, incapable of utilizing NH_3 at a significant rate. Owing to a lack of suitable fermentable substrate, excess ruminal NH_3 may be absorbed from the reticulo-rumen and recycled via the N pool (Leng & Nolan, 1984). A substantial portion of this N may eventually be excreted in the urine and therefore lost to the micro-organisms and the host (Satter & Roffler, 1975).

De Waal (1986) found that grazing lactating Merino and Dorper ewes exhibited lower rumen NH_3 levels than non-lactating ewes. The higher protein requirement posed for lactation could be responsible for this. In spite of a higher feed intake by lactating ewes (De Waal, 1986), drainage of N from the body pool for milk synthesis would have left less N available for recycling to the rumen, thereby accounting for the observed lower ruminal NH_3. Furthermore, the large differences in rumen NH_3 levels of the ewes in response to supplementary CP (E 0 *vs.* PE 0; Figures 7 to 10), compared to the small differences in animal performance (De Waal & Biel, 1989a), suggest that ruminal NH_3 had in fact not been the primary limiting factor in either rumen activity or animal production. Similar observations and conclusions for grazing Merino wethers were made by De Waal *et al.* (1989a; 1989b). If N *per se* had been the primary limiting factor, supplementary CP would have stimulated rumen activity and herbage intake. The results by De Waal *et al.* (1981), De Waal *et al.* (1989a) and De Waal & Biel (1989a; 1989b) show that this was not the case. These results substantiate the finding of De Waal & Biel (1989b) that the concept of supplements stimulating the rumen, especially in grazing sheep on the grassveld of the central Orange Free State, is questionable.

Acknowledgements

The authors are indebted to Dr E.A.N. Engels, Mrs Heila Terblanche, Dr Marion Read, Miss Maksie Baard and the late Dr Daan Els for valuable assistance rendered during the course of the trial.

References

ARMSTRONG, D.G. & SMITHARD, R.R., 1979. The fate of carbohydrates in the small and large intestines of the ruminant. *Proc. Nutr. Soc.* 38, 283.

BLACKBURN, T.H., 1965. Nitrogen metabolism in the rumen. In: Physiology of digestion in the ruminant. Ed. Dougherty, R.W., Butterworths, Washington, DC., p. 322.

BLOOMFIELD, R.A., KEARLEY, E.O., CREACH, D.O. & MURHER, M.E., 1963. Ruminal pH and absorption of ammonia and VFA. *J. Anim. Sci.* 22, 833.

BRYANT, M.P. & ROBINSON, I.M., 1963. Apparent incorporation of ammonia and amino acid carbon during growth of selected species of ruminal bacteria. *J. Dairy Sci.* 46, 150.

CHALUPA, W., 1975. Rumen bypass and protection of proteins and amino acids. (Symposium: Protein and amino acid nutrition in the high producing cow.) *J. Dairy Sci.* 58, 1198.

CHALUPA, W., 1984. Discussion of protein symposium. (Symposium: Protein nutrition of the lactating dairy cow.) *J. Dairy Sci.* 67, 1134.

CHALUPA, W., CLARK, J., OPLIGER, P. & LAVKER, R., 1970. Ammonia metabolism in rumen bacteria and mucosa from sheep fed soy protein or urea. *J. Nutr.* 100, 161.

CHURCH, D.C., 1973. In: Digestive physiology and nutrition of ruminants. Part I. Digestive Physiology. O.S.U. Book Stores Inc.

COETZEE, C.G. & LESCH, S.F., 1969. 'n Verdere studie van die konsentrasie en samestelling van vlugtige vetsure in

die rumen van skape op doodgerypte veldgrashooi. *Agroanimalia* 1, 175.

COETZEE, C.G., LESCH, S.F. & NEL, J.W., 1967. Geelmielies as aanvulling tot doodgerypte veldgras met verwysing na ruminale benutting van vismeel en ureum. *S.-Afr. Tydskr. Landbouwet.* 10, 1037.

DE WAAL, H.O., 1986. Die voedingswaarde van veldweiding van die sentrale Oranje-Vrystaat vir lakterende skape met spesiale verwysing na die rol van aanvullende energie en ruproteïen. Ph.D (Agric.)-proefskrif, Universiteit van Stellenbosch.

DE WAAL, H.O., BAARD, MARGARIETHA A. & ENGELS, E.A.N., 1989a. Effects of sodium chloride on sheep. 1. Diet composition, body mass changes and wool production of young Merino wethers grazing native pasture. *S. Afr. J. Anim. Sci.* 19, 27.

DE WAAL, H.O., BAARD, MARGARIETHA A. & ENGELS, E.A.N., 1989b. Effects of sodium chloride on sheep. 2. Voluntary feed intake and changes in certain rumen parameters of young Merino wethers grazing native pasture. *S. Afr. J. Anim. Sci.* 19, 34.

DE WAAL, H.O. & BIEL, L.C., 1989a. Supplementation of lactating Dorper and Merino ewes on *Themeda cymbopogon* veld. 1. Body mass changes of ewes and their lambs. *S. Afr. J. Anim. Sci.* 19, 141.

DE WAAL, H.O. & BIEL, L.C., 1989b. Supplementation of lactating Dorper and Merino ewes on *Themeda cymbopogon* veld. 2. Diet quality and feed intake. *S. Afr. J. Anim. Sci.* 19, 148.

DE WAAL, H.O., ENGELS, E.A.N. & VAN DER MERWE, F.J., 1980. Supplementing sheep with protein and phosphorus on native pasture of the central Orange Free State. 1. Diet composition, digestibility and rumen ammonia concentration. *S. Afr. J. Anim. Sci.* 10, 203.

DE WAAL, H.O., ENGELS, E.A.N., VAN DER MERWE, F.J. & BIEL, L.C., 1981. Supplementing sheep with protein and phosphorus on native pasture of the central Orange Free State. 2. Feed intake, mass changes and wool production. *S. Afr. J. Anim. Sci.* 11, 217.

DRORI, D. & LOOSLI, J.K., 1961. Urea and carboydrates versus plant protein for sheep. *J. Anim. Sci.* 20, 233.

DU PLESSIS, G.F. & VAN DER MERWE, F.J., 1970. Studies on the rumen metabolism of sheep fed freshly frozen lucerne or grazing lucerne pastures. I. Effect of carbohydrate supplementation on the pH of the rumen fluid. *Agroanimalia* 2, 7.

EL-SHAZLY, K., DEHORITY, B.A. & JOHNSON, R.R., 1961. Effect of starch on the digestion of cellulose *in vitro* and *in vivo* by rumen micro-organisms. *J. Anim. Sci.* 20, 268.

HARVEY, W.R., 1976. Least-squares and maximum likelihood general purpose program. (252 K Mixed Model Version). Rev. edn., Ohio State University.

HENNING, PENELOPE A., VAN DER LINDEN, YVONNE, MATTHEYSE, MARY E., NAUHAUS, W.K., SCHWARTZ, HELEN M. & GILCHRIST, FRANCES M.C., 1980. Factors affecting the intake and digestion of roughage by sheep fed maize straw supplemented with maize grain. *J. agric. Sci., Camb.* 94, 565.

HOGAN, J.P., 1975. Quantitative aspects of nitrogen utilization in ruminants. *J. Dairy Sci.* 58, 1164.

HOGAN, J.P. & WESTON, R.H., 1970. Quantitative aspects of microbial protein synthesis in the rumen. In: Physiology of digestion and metabolism in the ruminant. Ed. Phillipson, A.T. Oriel Press, Newcastle-upon-Tyne, England, p. 474.

HYND, P.I., 1984. Effects of starch fermentation products on roughage digestion. *J. agric. Sci., Camb.* 103, 469.

KARR, M.R., LITTLE, C.O. & MITCHELL, G.E., 1966. Starch disappearance from different segments of the digestive tract of steers. *J. Anim. Sci.* 25, 652.

KENNEDY, P.M., 1980. The effects of dietary sucrose and the concentrations of plasma urea and rumen ammonia on the degradation of urea in the gastrointestinal tract of cattle. *Br. J. Nutr.* 43, 125.

LENG, R.A., 1970. Formation and production of volatile fatty acids in the rumen. In: Physiology of digestion and metabolism in the ruminant. Ed. Phillipson, A.T. Oriel Press, Newcastle-upon-Tyne, England, p. 406.

LENG, R.A., 1984. Supplementation of tropical and subtropical pastures for ruminant production. In: Herbivore nutrition in the subtropics and tropics. Eds. Gilchrist, F.M.C. & Mackie, R.I. The Science Press, Craighall, RSA, p. 129.

LENG, R.A. & NOLAN, J.V., 1984. Nitrogen metabolism in the rumen. (Symposium: Protein nutrition of the lactating dairy cow.) *J. Dairy Sci.* 67, 1072.

LOUW, G.N., 1978. Die aanwending van voedselhulpbronne vir diereproduksie. *S.-Afr. Tydskr. Veek.* 8, 79.

MACKIE, R.I., GILCHRIST, FRANCES M.C., ROBBERTS, P.E. HANNAH & SCHWARTZ, HELEN M., 1978. Microbiological and chemical changes in the rumen during the stepwise adaptation of sheep to high concentrate diets. *J. agric. Sci., Camb.* 90, 241.

MACLEOD, N.A., ØRSKOV, E.R. & ATKINSON, T., 1984. The effects of pH on the relative proportions of ruminal volatile fatty acids in sheep sustained by intragastric infusions. *J. agric. Sci., Camb.* 103, 459.

MANN, S.O. & ØRSKOV, E.R., 1975. The effect of feeding whole or pelleted barley to lambs on their rumen bacterial population and pH. *Proc. Nutr. Soc.* 34, 63.

MARTIN, A.K. & BLAXTER, K.L., 1965. The energy cost of urea synthesis in sheep. In: Energy metabolism. Ed. Blaxter, K.L. London Academic Press.

MEHREZ, A.Z. & ØRSKOV, E.R., 1978. Protein degradation and optimum urea concentration in cereal-based diets for sheep. *Br. J. Nutr.* 40, 337.

OBARA, Y. & SHIMBAYASHI, K., 1980. The appearance of re-cycled urea in the digestive tract of goats during the final third of a once daily feeding of a low-protein diet. *Br. J. Nutr.* 44, 295.

OLSON, J.C., CRAMER, D.A. & NAGY, J.G., 1968. Gastrointestinal pH values of sheep by breed. *J. Anim. Sci.* 27, 1117 (Abstr. 56).

ØRSKOV, E.R., 1975. Manipulation of rumen fermentation for maximum food utilization. *Wld. Rev. Nutr. Diet.* 22, 152 (Karger, Basel).

ØRSKOV, E.R., 1977. Nitrogen digestion and utilization by young and lactating ruminants. *Wld. Rev. Nutr. Diet.* 26, 225 (Karger, Basel).

ØRSKOV, E.R., 1982. Maintenance and growth in ruminants. In: Energy metabolism of farm animals. *Proc. 9th Symposium,* Lillehammer, Norway, p. 141.

ØRSKOV, E.R., FRASER, C. & KAY, R.N.B., 1969. Dietary factors influencing the digestion of starch in the rumen and small and large intestine of early weaned lambs. *Br. J. Nutr.* 23, 217.

PRITCHARD, R.H. & MALES, J.R., 1982. Effects of supplementation of wheat straw diets twice a day on rumen ammonia, volatile fatty acids and cow performance. *J. Anim. Sci.* 54, 1243.

REED, W.D.C., ELLIOTT, R.C. & TOPPS, J.H., 1968. Effects of level of intake and roughage content of cattle diets on digestibility, urinary energy and mineral losses, and certain aspects of rumen function. *Rhod. J. agric. Res.* 6, 81.

SATTER, L.D. & ROFFLER, R.E., 1975. Nitrogen requirement and utilization in dairy cattle. *J. Dairy Sci.* 58, 1219.

SATTER, L.D. & SLYTER, L.L., 1974. Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nutr.* 32, 199.

SCHAEFER, D.M., DAVIS, C.L. & BRYANT, M.P., 1980. Ammonia saturation constants for predominant species of rumen bacteria. *J. Dairy Sci.* 63, 1248.

STERN, M.D. & HOOVER, W.H., 1979. Methods for determining and factors affecting rumen microbial protein synthesis: a review. *J. Anim. Sci.* 49, 1590.

SWART, J.A., NIEMANN, P.J., ENGELS, E.A.N. & BIEL, L.C., 1971. The influence of fish meal and yellow maize on the nutritive value of *Themeda triandra* for Merino sheep. *S. Afr. J. Anim. Sci.* 1, 25.

TERRY, R.A., TILLEY, J.M.A. & OUTEN, G.E., 1969. Effect of pH on cellulose digestion under *in vitro* conditions. *J. Sci. Fd. Agric.* 20, 317.

TUCKER, R.E., MITCHELL, G.E. & LITTLE, C.O., 1968. Ruminal and postruminal starch digestion in sheep. *J. Anim. Sci.* 27, 824.

VAN NIEKERK, A.I. & VAN DER MERWE, F.J., 1966. Some aspects of the nitrogen metabolism of sheep with special reference to the rumen. *Proc. S. Afr. Soc. Anim. Prod.* 5, 61.

WALKER, D.J. & NADER, C.J., 1970. Rumen microbial protein synthesis in relation to energy supply: diurnal variation with once-daily feeding. *Aust. J. agric. Res.* 21, 747.

WARNER, A.C.I., 1965. Factors influencing numbers and kinds of microorganisms in the rumen. In: Physiology of digestion in the ruminant. Ed. Dougherty, R.W. Butterworths, Washington, DC, p. 346.

WESTON, R.H. & HOGAN, J.P., 1968. Rumen ammonia in relation to characteristics of the diet and parameters of nitrogen metabolism. *Proc. Aust. Soc. Anim. Prod.* 7, 359.