SUPPLEMENTATION OF CEREAL STRAWS WITH LUCERNE AND SUGAR BEET PULP IN DIETS FOR RUMINANTS

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DECLARATION

I declare that this thesis is my own composition, and does not include work submitted for any other degree or professional qualification. The experimental work reported here was planned, carried out and analized by myself with help from members of staff of the Edinburgh School of Agriculture and the East of Scotland College of Agriculture.

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LIST OF ABBREVIATIONS

ADF = acid detergent fibre
ADL = acid detergent lignin
ANOVA = analysis of variance
ATP = adenosine-tri-phosphate
BP = sugar beet pulp
°C = degrees Celsius
CF = crude fibre
cm = centimetre
CP = crude protein
CV = coefficient of variation
c.v. = cultivar, variety
DM = dry matter
DMD = dry matter digestibility
DMI = dry matter intake
DOMD = digestible organic matter in the dry matter
e.g. = for example
g = gramme
GE = gross energy
h = hour(s)
i.e. = that is
i.u. = international unit(s)
J = joule (0.239 calories)
k = kilo (thousand)
l = litre
L = Lucerne
LW = live weight
LWG = live weight gain
m = metre or milli (10^{-3})
MADF = modified acid detergent fibre
ME = metabolizable energy
MF = modulus of fineness
min = minutes
mol = mole
n = number of replicates or samples
N = nitrogen
NDF = neutral detergent fibre
NH_3 = ammonia
NPN = non-protein nitrogen
NS = non significant
OM = organic matter
OMD = organic matter digestibility
OMI = organic matter intake
pH = logarithmic index of hydrogen ion concentration
r = correlation coefficient
RDP = rumen degradable protein
r.s.d. = residual standard deviation
s.d. = standard deviation
s.e. = standard error
$s.e._{mean(s)}$ = standard error of a(the) mean(s)
$s.e._{diff.}$ = standard error of differences of means
TS = treated straw
µ = micro (10^{-6}), micrometre

UDP	= rumen-undegradable protein
US	= untreated straw
VFA	= volatile fatty acids
v.	= versus
*	= probability (P) $<$ 0.05
**	= P $<$ 0.01
***	= P $<$ 0.001

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ABSTRACT

The aim of this work was to study the effects of supplementing untreated (US) and ammonia-treated (TS) barley straw with lucerne (L) and/or unmolassed sugar beet pulp (BP) on the digestibility and intake of the dietary components in ruminants. Coarsely shredded straw was fed *ad libitum* and pelleted supplements at restricted levels.

Rumen-fistulated steers and wethers were offered a diet containing (g/kg diet, DM basis): 760 US and 240 L. The *in sacco* degradation of US and L, estimated with the dacron bag technique, was similar for both species at most incubation times (5, 8, 11, 14, 24, 48 and 72 h). The differences for pH, ammonia-nitrogen (NH_3-N) and volatile fatty acids (VFA) in the rumen were small and inconsistent among times post-feeding. The daily straw organic matter intake (OMI, per kg $LW^{0.75}$ or $LW^{0.9}$) was higher in steers than in wethers.

In two experiments, mature rumen-fistulated wethers were offered either US or TS supplemented with L at levels of (g/kg diet, DM basis): 0, 160, 320 and 480. These diets were also fed to 35-kg wethers in two digestibility and intake trials. Increasing the level of lucerne had little or no effects on the *in sacco* degradation of straw and lucerne nor on most parameters of rumen fermentation. The *in vivo* digestibility and metabolizable energy (ME) of the diets and the N retained by the sheep increased linearly as the level of lucerne increased. The organic matter digestibility (OMD) and ME for US and TS fed alone were 0.40 and 0.60 and 5.1 and 8.5 MJ per kg DM, respectively. There was little or no replacement of the straws by lucerne.

Comminution and passage of chromium-mordanted straws were studied with mature rumen-fistulated wethers offered four diets: US and TS alone or supplemented with L at 480 g per kg diet, DM basis. The rates of comminution of large straw particles (retained on sieves with aperture size > 1.18 mm) in the rumen were similar among treatments (0.055 per h). The rate of escape of small straw particles (passing through a 1.18-mm sieve) from the rumen was faster for the supplemented TS (0.033 per h) than for the other diets (0.021 per h).

In two experiments, mature rumen-fistulated wethers were offered either US and pellets at 320 g per kg diet, DM basis or TS and pellets at 480 g per kg diet, DM basis. The pellets contained L:BP in the following relative proportions: 3:0 (US-0BP and TS-0BP); 2:1 (US-33BP and TS-33BP); 1:2 (US-67BP and TS-67BP) and 0:3 (US-100BP and TS-100BP). The diets were also fed to 35-kg wethers in two digestibility and intake trials. Although the differences were mostly non-significant, rumen pH and NH_3-N tended to decrease while total rumen VFA tended to increase as the level of BP increased. The *in sacco* degradation of straw tended to be higher for diet US-100BP than for diet US-0BP. It tended to increase between diets TS-0BP and TS-67BP and then to decrease for diet TS-100BP. There were indications of positive associative effects of L and BP (1:2 ratio) on the intake and/or digestibility of the total diet. The US-OMI increased linearly whereas the TS-OMI remained constant as the level of BP increased.

It was concluded that lucerne and sugar beet pulp were effective supplements for barley straws as they generally maintained or increased the digestibility and/or intake of the straws and also increased significantly the intake of digestible nutrients.

CHAPTER 1

GENERAL INTRODUCTION

Production of food for the human population is and will be of great concern worldwide. It has been predicted that the deficit of major staple foods in developing countries in the 1990's will be almost four times the deficit in the late 1970's (See Brady, 1981). Because of this and limitations in availability and cost of many agricultural inputs, it is almost certain that the production of animal and vegetable foods will increase, with a greater efficiency in the use of resources. In this regard, ruminants will play an important rôle. When properly evaluated (Engelhardt, Dellow and Hoeller, 1985; Spedding, 1984), ruminants are highly efficient converters of forages, waste and fibrous by-products into valuable human foods. In addition, ruminants can provide many non-food products and services to man which are often ignored when assessing their biological efficiency (McDowell, 1980).

Throughout the world there are large amounts of fibrous feeds which are of little or no use to man as food. About a quarter of the world's land surface is permanent pasture and about a third is forest and woodland (Food and Agriculture Organization, FAO, 1984). Approximately half of the biomass from permanent and seasonal crops is of no direct use as human food. The potential of ruminants, and other herbivores, for the production of valuable foods for man is therefore enormous, but not yet fully exploited.

Between 1970 and 1981 the world production of fibrous by-products from cereal and other crops increased by 36 %. The population of "grass eaters" (livestock units of horses, mules, asses, cattle, buffaloes, sheep, goats and camels) increased only by 2.1 and 13.3 % in developed and most developing countries, respectively. On the other hand, the respective increases of "grain

eaters" (livestock units of pig and poultry) were 17.5 and 38.5 % (Kossila, 1984). An increase of 2.2 % per year in the use of grains for animal feeding in the world was expected to occur between the middle of the past and present decades (Dale, 1979). Throughout the world emphasis has been given to the production of food of animal origin from non-ruminants consuming grains. In developed countries, large amounts of grain are fed to ruminants as well. In developing countries such an approach is often justified economically on the basis of the individual enterprise, but seldom on a national basis; in developed countries it is justified in both cases. These trends are not likely to change substantially in the future as they are strongly supported by economic reasons and seldom shaken by moral principles. Therefore, a pragmatic approach should be used in developing countries *i.e.* to give emphasis to the improvement of the utilization of available resources such as fibrous feeds and the ruminant and to optimize the use of the scarce and expensive feeds such as cereal and oilseed grains.

There is still great scope to improve the utilization of roughages by ruminants, particularly in tropical areas. The nutritive value of grasses can be improved by introducing a legume in a native pasture or by planting an improved grass-legume mix ('tMannetje, 1984). Where this not fully achievable for climatic and socio-economic reasons, leguminous trees and shrubs whose foliage can be browsed or harvested are a promising alternative (Ibrahim, 1981; National Academy of Sciences, 1979). The harvested foliage could be used as a supplement for low-quality roughages. Effective supplementation with these and other feeds could be achieved by promoting optimum degradation of plant cell walls in the rumen. This would require feeding of rumen degradable protein, good quality fibre (*e.g.* sugar beet pulp), minerals and other factors apparently provided by green forage (Preston and Leng, 1984). For levels of

production higher than those expected when rumen fermentation is maximized, upgrading of the basal roughage and/or supplementation with feeds such as grains and sources of rumen undergradable protein would be required.

The aim of the work reported in this thesis was to produce results applicable to tropical areas. Three basic principles underlie the design of the experiments: a) minimizing the energy costs in the preparation and/or upgrading of low-quality roughages; b) promoting favourable conditions for an optimum rumen fermentation; c) maximum use of feeds of little or no value for feeding non-ruminants, including man. The aims of the present work were: a) to study the rumen digestion and intake of a straw-lucerne diet by steers and wethers; b) to evaluate the effects of supplementing untreated and ammonia-treated straw with lucerne and/or sugar beet pulp in diets for sheep on:

-the *in sacco* degradation of the dietary components in the rumen
-some parameters of rumen fermentation
-rates of comminution and passage of straw particles in the rumen
-the *in vivo* digestibility and intake of the diets.

A review of the related literature is presented in Chapter 2. In Chapter 3, a comparative study of the rumen digestion and intake of a straw-lucerne diet by steers and wethers is presented. Work on the use of lucerne as a supplement to barley straws in diets for sheep is described in Chapter 4. Aspects of the rates of comminution of straw particles in the rumen and their passage to the hindgut in sheep receiving straw-lucerne diets were studied in the experiment described in Chapter 5. The effects of replacing lucerne by sugar beet pulp in supplements for barley straws on the digestibility and intake of the diets by sheep are dealt with in Chapter 6. The results are

discussed in Chapter 7 in relation to the aims of this work and the potential of the diets for ruminant animal production.

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

Low-quality roughages form a heterogeneous group of plant materials which are high in lignified fibre and low in nitrogen and some minerals. This group includes: range plants (grasses and browse); straws (the crop residues left after grain harvesting) and the by-products of industrial processing of plant materials (Balch, 1977). This review is focussed on the crop residues of most economic importance worldwide; *i.e.* barley, oat, rice and wheat straws. Relevant studies with other low-quality roughages are also discussed.

The major energy-yielding substrates in cereal straws, cellulose and hemicellulose, are contained in the cell walls which represent up to 0.80 of the total dry matter (Preston and Leng, 1984; Theander and Åman, 1984). Unlike the hosts themselves, microorganisms in the gastrointestinal tract of herbivores produce enzymes to degrade cellulose and hemicelluloses. Herbivores consuming fibrous diets rely on the end-products of microbial fermentation for their supply of energy. With a substantial foregut microbial fermentation, ruminants can also obtain energy and other nutrients through enzymic digestion of microbial matter and feed reaching the hindgut.

In straws, the digestion of cellulose and hemicelluloses by gastrointestinal microorganisms is hindered by several inherent characteristics of the cell walls (Cowling and Kirk, 1976). As a result, the energy that ruminants can obtain from straws is, in most cases, insufficient for their maintenence requirements. There are two main approaches to help overcome this problem: chemical, physical or biological treatment of straw and supplementation of straw-based diets with more digestible feeds.

In this chapter, several aspects of the characteristics of plant cell walls and their digestion in the rumen are reviewed, followed by sections on the use of highly-digestible fibrous feeds as supplements to low-quality roughages in diets for ruminants.

2.2. BOTANICAL AND CHEMICAL CHARACTERISTICS OF PLANT CELL WALLS

From a botanical point of view, cell wall is a dynamic material arranged as a multi-layered flexible structure with two basic components: a disperse phase of microfibrils and a complex continuous matrix between these fibres (Juniper, 1979 ; Northcote, 1972). The major organic constituents of the plant cell wall are: cellulose, hemicellulose, pectin and lignin; with phenolic acids, bound proteins, gums, mucilages, waxes, cutin and suberin in minor concentrations (Bailey, 1973; Juniper, 1979; Laplace and Lebas, 1981; Northcote, 1972). Cellulose makes up the microfibrillar phase in most plants. It is a single polymer of β(1-4) linked D-glucoses with cellobiose as the repeating unit (Bailey, 1973). The matrix component contains water, polysaccharides (hemicelluloses and pectic substances) and lignin as the major fractions. Water is a highly variable feature of the cell wall having several functions related to the strength of the whole structure and its permeability (Northcote, 1972). Pectic substances are a complex mixture of amorphous polysaccharides serving as an intercellular cement (Bailey, 1973; Esau, 1965). They are essentially a polymer of α(1-4) linked galacturonic acids with araban and possibly galactan side chains (Van Soest, 1982). Hemicelluloses have not been exactly defined. Generally, they are a mixture of glycans composed of pentose or hexose units dominated by linear chains of β(1-4) linked D-xylopiranose units with branches of arabino-, gluco- and/or galactopiranosides (Akin and Barton, 1983; Bailey, 1973).

Lignin is a condensed three-dimensional polymer of phenylpropane units, which can be classified into three groups: p-coumaryl alcohols and p-coumaric acids; coniferyl alcohols and ferulic acids and sinapyl alcohols (Theander and Åman, 1984; Van Soest, 1982). These are interconnected in such a variety of proportions and sequences and with such irregularly ocurring chemical bonds that the description and isolation of true lignin are far from exact (Harkin, 1973). While giving structural support to plant cell walls, lignin easily bonds with a wide range of other components (Juniper, 1979). Of the phenylpropane units present in lignin, p-coumaric and ferulic acids are also bound to various chemical fractions of the cell solubles (Harris and Hartley, 1976; Jung, Fahey and Garst, 1983).

In graminaceous species of nutritional interest, a thin layer of cutin and wax, namely cuticle, covers the external walls of epidermal cells and the entire surface of various plant parts. Both cutin and wax are formed mainly of fatty acids and cutin is a structural polymer. Cuticle helps to control water exchange between plant cell walls and the enviroment and adds rigidity and strength to vegetable tissues (Martin and Juniper, 1970).

Plant cell walls contain small amounts of nitrogen; mostly associated with lignin. Its exact origin is not known, but it seems to be related to Maillard polymers and extensin, a glycoprotein associated with the fibrillar component which is likely to be nutritionally unavailable due to lignin incrustation (Bailey, 1973; Goering, Gordon, Hemken, Waldo, Van Soest and Smith, 1972; Northcote, 1972; Van Soest, 1982).

2.3. DIGESTION OF CELL WALLS BY THE RUMINANT

2.3.1. Biochemical pathways

Molecules of high energy concentration which are required for the biochemical reactions in the microbial metabolism result from the microbial anaerobic fermentation of cell walls in the rumen. Volatile fatty acids (VFA, primarily acetic, propionic and butyric), carbon dioxide and methane are produced as waste products from these reactions. VFA represent the major source of energy for the ruminant (Hungate, 1966, 1975). In addition, the host is provided with bacterial cells; a source of energy, protein and other nutrients after enzymic digestion in the hindgut. Lactate, succinate, formate and hydrogen are intermediate- extracellular products of the fermentation reactions. Lactate is largely converted to propionate and does not accumulate under normal conditions in the rumen. Formate is rapidly metabolized into carbon dioxide and methane and succinate is a precursor of most of the propionate in the rumen. Hydrogen is mostly used to reduce carbon dioxide to methane (Hungate,1966, 1975; Wolin and Miller, 1983). These metabolic conversions are summarized in Figure 2.1. For detailed accounts of the metabolic pathways and stoichiometry of anaerobic fermentation of carbohydrates in the rumen see Buttery (1977) and Hungate (1966).

2.3.2. Microbial digestion

Rumen bacteria vary in their ability and versatility to hydrolyze the different components of the cell walls and the most active and predominant species capable of degrading cellulose, hemicellulose and/or pectin substances have been identified. *Bacteroides succinogenes*, *Ruminococcus flavefaciens* and *Ruminococcus albus* are the most effective cellulolytic bacteria in the rumen of cattle and sheep, with some strains of *Butyrivibrio fibrisolvens* having

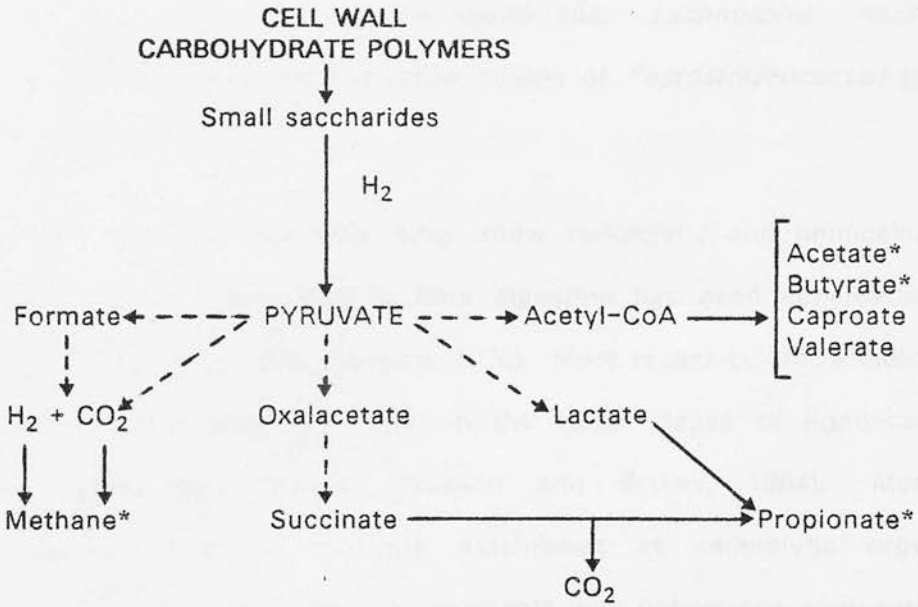

FIGURE 2.1 Summary of biochemical pathways in the anaerobic fermentation of cell wall carbohydrates. Dashed lines: intermediate pathways. * : terminal products that accumulate in the rumen. (Adapted from Russell and Hespell, 1981; Wolin and Miller, 1983).

limited ability to degrade cellulose . Of these, *B. succinogenes* and *R. flavefaciens* can hydrolyze closely aligned cellulose whereas *R. albus* can degrade only randomly aligned cellulose or its soluble products (Bryant, 1973; Bryant and Small, 1956; Gows and Kistner, 1965; Stewart, Dinsdale, Chang and Paniagua, 1979). The principal hemicellulose digesters are *B. ruminicola, B. fibrisolvens, Eubacterium ruminantium* and certain strains of ruminococci (Bryant, 1973; Dehority, 1973; Hungate, 1966). Pectic substances are predominantly degraded by *B. fibrisolvens, Lachnospira multiparus, Succinivibrio dextrinisolvens* and some strains of *Peptostreptococcus* (Bryant, 1977; Dehority, 1973).

Rumen protozoa and anaerobic fungi show cellulolytic and hemicellulolytic activity, but their contribution to fibre digestion has been considered very small (Amos and Akin, 1978; Hungate, 1975). More recent evidence indicates a significant rôle of anaerobic fungi in the initial stages of ligno-cellulose digestion (Bauchop, 1979a,b; Chesson and Ørskov, 1984). Moreover, entodinomorph protozoa are now established as cellulolytic organisms (Coleman, 1985; Demeyer, 1981). Experiments with defaunated ruminants have shown that the presence of protozoa accounts for about a third of the total microbial fibre digestion in the rumen. This may be due to protozoal cellulolytic activity and to indirect effects of protozoa on the activity and relative proportions of cellulolytic bacteria (Demeyer, 1981).

The breakdown of long forages in ruminants is started by mastication. This helps to release soluble components for immediate use by rumen microorganisms and increases the surface area and degree of cell wall damage which favour microbial attachment (Doyle, 1967; Hogan, 1965; Pond, Ellis and Akin, 1984). With the exception of *R. albus*, fibre-digesting bacteria do not

secrete significant amounts of soluble extracellular enzymes and, therefore, they must adhere to or be near plant cell walls before enzymic digestion occurs (Akin, 1979; Akin and Barton, 1983; Dehority, 1973; Leatherwood, 1973; Smith, Yu and Hungate, 1973). Indeed, *R. flavefaciens* secretes an adhesive glycoprotein coat closely associated with degrading cell walls. Moreover, bacterial cellulases are present close to the bacteria or adsorbed to cellulose fibres, with very small amounts free in the rumen fluid (Cheng and Costerton, 1980; Gawthorne, 1979; Latham, Brooker, Pettipher and Harris, 1978; Leatherwood, 1973). While *B. succinogenes* adheres strongly to damaged plant material *R. albus* shows limited attachment capacity (Chesson and Ørskov, 1984; Patterson, Irvin, Costerton and Cheng, 1975).

Degradation of cellulose and hemicellulose without bacterial attachment has been demostrated also, but its contribution to total cell wall digestion is still uncertain. Reports on the activity of cell-free cellulases in the rumen are conflicting (Akin, 1982).

2.4. FACTORS AFFECTING CELL WALL DIGESTION IN THE RUMEN

The practical definition of nutritional fibre for the ruminant has been limited to the insoluble fraction in the plant cell walls which includes cellulose, hemicellulose, lignin, cutin, and bound protein (Van Soest, 1985). Hereafter, the term fibre will be used in this context.

Many interacting factors influence the digestion of fibre in the gastrointestinal tract of herbivores. These include: physiological factors in the host, dietary factors and microbial factors. Fibre digestion in ruminants will be discussed as outlined by Mertens (1977) and Mertens and Ely (1982). According to them, the digestion of fibre in the rumen is affected by four components: lag phase or the time before the onset of bacterial fermentation; rate of digestion;

potential extent of digestion and rate of passage. Chemical and physical characteristics of the fibre, microbial activity, factors related to the physiology of the host and their interactions affect one or more of these four components (Figure 2.2).

2.4.1. Chemical characteristcs of fibre

The extent of digestion of fibre is influenced by several chemical fractions in the cell wall. Of these, lignin and its derivates, silica and cutin are the most important.

Generally, the digestibility of OM in forages is negatively correlated with lignin. However, the degree of correlation is highly influenced by plant species, methods of lignin analysis and type and strength of lignin–polysaccharide bondings (Barton and Akin, 1977; Jung *et al*, 1983; Minson, 1982). Three mechanisms have been proposed to explain the negative effects of lignin on OM digestibility: a) physical protection of cell wall components preventing microbial attack; b) inhibition of microbial enzymic activity and c) development of strong lignin–polysaccharide bondings (Dehority and Johnson, 1961; Harkin, 1973; Juniper, 1979; Morrison, 1973; Van Soest and McQueen, 1973). More recently, it has been shown that phenolic acids have antimicrobial properties and that they could be the main link between lignin and cell wall structural carbohydrates (Akin, 1982; Hartley, 1973; Jung and Fahey, 1983). Whereas there is full agreement on the negative effects of lignin on the extent of forage digestion (Mertens and Ely, 1982; Minson, 1982; Smith, Goering and Gordon, 1972), reports on the effects of lignin on digestion rate are conflicting. Smith *et al* (1972) and Mertens (1977) related lignin content to the digestion rate of potentially digestible cell wall (1 – indigestible residue after 72 h of incubation). In most forages, lignin is of very low digestibility and most of it

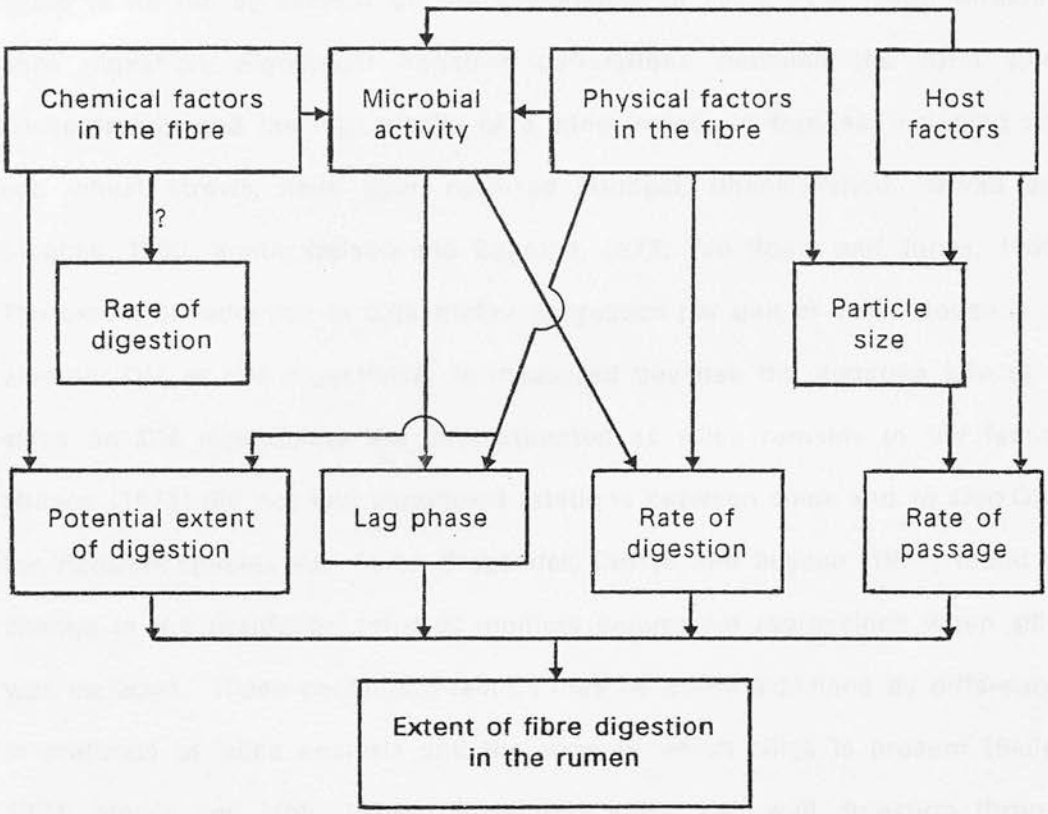

FIGURE 2.2 Factors affecting the digestion of fibre in the rumen. (Adapted from Mertens, 1977 and Mertens and Ely, 1982).

remains in the indigestible residue (Barsaul and Talapatra, 1971). Thus, lignin content and digestion rate of potentially digestible cell walls are likely to be poorly correlated. Moreover, the high variation in cellulose digestibility by ruminants is attributed not only to differences in lignin concentration but also to silica and cutin concentrations (Van Soest, 1973).

There is no full agreement on the importance of silica as a factor affecting fibre digestion. Significant negative correlations between the total silica concentration and the digestibility of a wide variety of forages, including rice and wheat straws, have been reported (Mudgal, Dhanalakshmi, Nawab and Singhall, 1980; Smith, Nelson and Boggino, 1971; Van Soest and Jones, 1968). The extent of reduction in digestibility, expressed per unit of silica, depends on whether DM or OM digestibility is measured because the negative effects of silica on DM digestibility are overestimated as silica remains in the faeces. Minson (1971) did not find significant relations between silica and *in vivo* OMD for *Panicum* species and Aerts, Brabander, Cottyn and Buysse (1977) found no change in the prediction error of multiple summative regressions when silica was included. These conflicting results may be partly explained by differences in methods of silica analysis and the form in which silica is present (Bailey, 1981; Moore and Moh, 1973). Silica may affect cell wall digestion through incrustation of fibre and inhibition of microbial cellulolytic activity by soluble silica compounds (Harbes, Raiten and Paulsen, 1981; Smith *et al*, 1971; Van Soest and Jones, 1968). Furthermore, the presence of epidermal silica hinders the physical rupture of cuticular tissues (Harbers *et al*, 1981).

Fibre digestion is also impaired by the presence of cuticle on the epidermis of individual cells and the surface of leaves and stems. Rumen bacteria cannot degrade cuticle and they degrade plant tissues only through cut edges and

ruptured surfaces resulting from fibre comminution (Akin and Amos, 1975; Brazle, Harbers and Owensby, 1979; Harbes *et al*, 1981; Monson, Powell and Burton, 1972).

2.4.2. Physical characteristics of fibre

Total fibre digestion may be affected by particle size and the physical characteristics of the fibre through changes in digestion rate, lag phase, potential extent of digestion and rate of passage (Figure 2.2).

Generally, *in vitro* digestibility of the cell walls increases as particle size decreases (Dehority and Johnson, 1961; Fan, Gharpuray and Lee, 1981; Pigden and Heaney, 1969; Stone, Scallen, Donefer and Ahlgren, 1969). The extent of this increase depends on the type of fibre, the method of mechanical processing and the degree of comminution (Fan et al, 1981; Robles, Belyea, Martz and Weiss, 1980). For example, whereas lucerne ground through 1- and 4-mm screens had higher digestion rate constants than those of lucerne ground through 8- and 12-mm screens (0.063 *v.* 0.040 per h)particle size did not affect rate constants of orchard grass (0.041 per h) (Robles *et al*, 1980). The beneficial effects of reducing particle size on digestibility *in vivo* are partly counteracted by the faster rate of passage of ground forages through the rumen which limits the time available for microbial attack and reduces fibre digestibility. For further information on the effects of grinding on the utilisation of forages see Greenhalgh and Wainman (1972); Minson (1963) and Owen (1978).

Crystallinity, water adsorption and ion exchange capacity also seem to affect the digestibility of forages (Chesson and Ørskov, 1984; Van Soest, 1982). Crystallinity values give an indication of the space distribution and the strength of association of cellulose molecules in the cell wall. Microbial

colonisation and cellulolysis on crystalline regions are slower than on amorphous regions in the cellulose (Cowling and Kirk, 1976; Fan *et al*, 1981; Smith *et al*, 1973). Crystallinity of isolated cellulose from cereal straws has been associated with the length of the lag phase while the relations with rate and extent of cellulose digestion are not well established (Chesson and Ørskov, 1984; Mertens and Ely, 1982).

Water adsorption and ion exchange can affect fibre digestion through their effects on the lag phase and the rate of passage. As the colonisation of newly ingested forage requires the movement of microorganisms via the rumen fluid to the arriving particles, a shorter lag phase may be associated with rapidly hydrating particles. It has been suggested that bacteria adhere to feed particles by ionic attraction (Van Soest and Sniffen, 1984). Therefore, ion exchange may affect the lag phase and even the rate of fibre digestion. Adsorption of water and ions leads to an increased specific gravity of forage particles (Hooper and Welch, 1985a,b) which has been related to a faster rate of passage of inert particles (Campling and Freer, 1962) and chromium-mordanted lucerne (Ehle, 1984).

2.4.3. Microbial growth and activity

With roughage-based diets it is necessary that the rumen environmental conditions and the supply of nutrients should be adequate at all times to achieve optimum microbial growth and activity. Only under such conditions can fibre digestion and the supply of microbial protein to the host be maximized. The effects of nutrients on growth and activity of rumen bacteria have been clearly identified under controlled *in vitro* conditions (See Bryant, 1973; Hespell and Bryant, 1979). The significance of these factors *in vivo* has not been always apparent, partly because of the extensive turnover of

microbial matter within the rumen (Nolan and Stachiw, 1979) and the many interactions of microbial species in substrate fermentation (Wolin, 1975; Wolin and Miller, 1983).

2.4.3.1. Rumen pH

The inhibitory effects of low pH (below 6–6.5) on the cellulolytic activity of fibre-digesting bacteria has been widely demonstrated by the addition of inorganic acids, starch and/or soluble sugars to *in vitro* cultures (Hiltner and Dehority, 1983; Russell and Dombrowski, 1980; Smith *et al*, 1973; Stewart, 1977; Stewart *et al*, 1979). Roughage intake and digestibility (*in vivo* and *in sacco*) decrease when cereal grains or molasses account for more than 0.15–0.40 of diets for sheep and cattle; this critical level increases as the quality of the basal roughage increases (Agricultural Research Council (ARC), 1980; Blaxter, Wainman and Wilson, 1961; Chimwano, Ørskov, Stewart and Grand, 1977; Fahmy, Lee and Ørskov, 1984; Miller and Muntifering, 1985; Mould, Ørskov and Mann, 1983; Williams, 1984). These negative effects are largely due to a lowered rumen pH induced by the acids from the rapid fermentation of soluble carbohydrates (Mould, Ørskov and Gauld, 1983; Mould *et al*, 1983; Prins and Clarke, 1980). However, other factors appear to be involved: repression of cellulases and hemicellulases by soluble sugars or by inhibitors secreted by sugar fermenters; competition between cellulolytic and noncellulolytic bacteria for essential nutrients, and increased lag phase in fibre digestion due to preferential use of soluble carbohydrates by some cellulolytic bacteria *per se* (El-Shazly, Dehority and Johnson, 1961; Mertens and Loften, 1980; Miller and Muntifering, 1985; Prins and Clarke, 1980; Smith *et al*, 1973).

2.4.3.2. Nutritional requirements of the rumen microbes

Microbial growth and activity in the rumen are always dependent on the availability of nutrients: fermentable energy, nitrogen, minerals, vitamins and growth factors (*e.g.* branched chain fatty acids). These nutrients are largely supplied by the diet with varying contributions from endogenous secretions, recycled nutrients and microbial turnover (Figure 2.3).

Generally, an inadequate supply of one of more nutrients results in impaired microbial digestion of substrates in the rumen and/or uncoupled fermentation. As a consequence substrate degradation and/or microbial growth are reduced (Demeyer, 1981; Hespell and Bryant, 1979). Recently, much attention has been given to the need for feeding strategies to supply frequent and proportional amounts of nutrients to the rumen microbes for their activity and growth to be maximized (ARC, 1984; Juul-Nielsen, 1981; Sniffen, Russell and Van Soest, 1983).

2.4.3.2.1 Fermentable energy

Most of the energy (ATP) for microbial growth in the rumen comes from the anaerobic fermentation of carbohydrates (See Figure 2.3). The rate of energy release depends on the fermentability of the carbohydrate source, which affects the growth and activity of rumen microbes (Stouthamer and Bettenhausen, 1973). The growth rate and the maintenence energy requirements of rumen bacteria are important factors determining the efficiency of utilisation of the available energy for growth. The effect of microbial relative growth rate (g DM synthesis per g microbial DM per h) on microbial growth efficiency (g microbial DM produced per mol ATP utilized) is illustrated in Figure 2.4, for three maintanence coefficients (mmol ATP per g microbial DM per h): low (cellulolytics);medium (mixed bacteria) and high

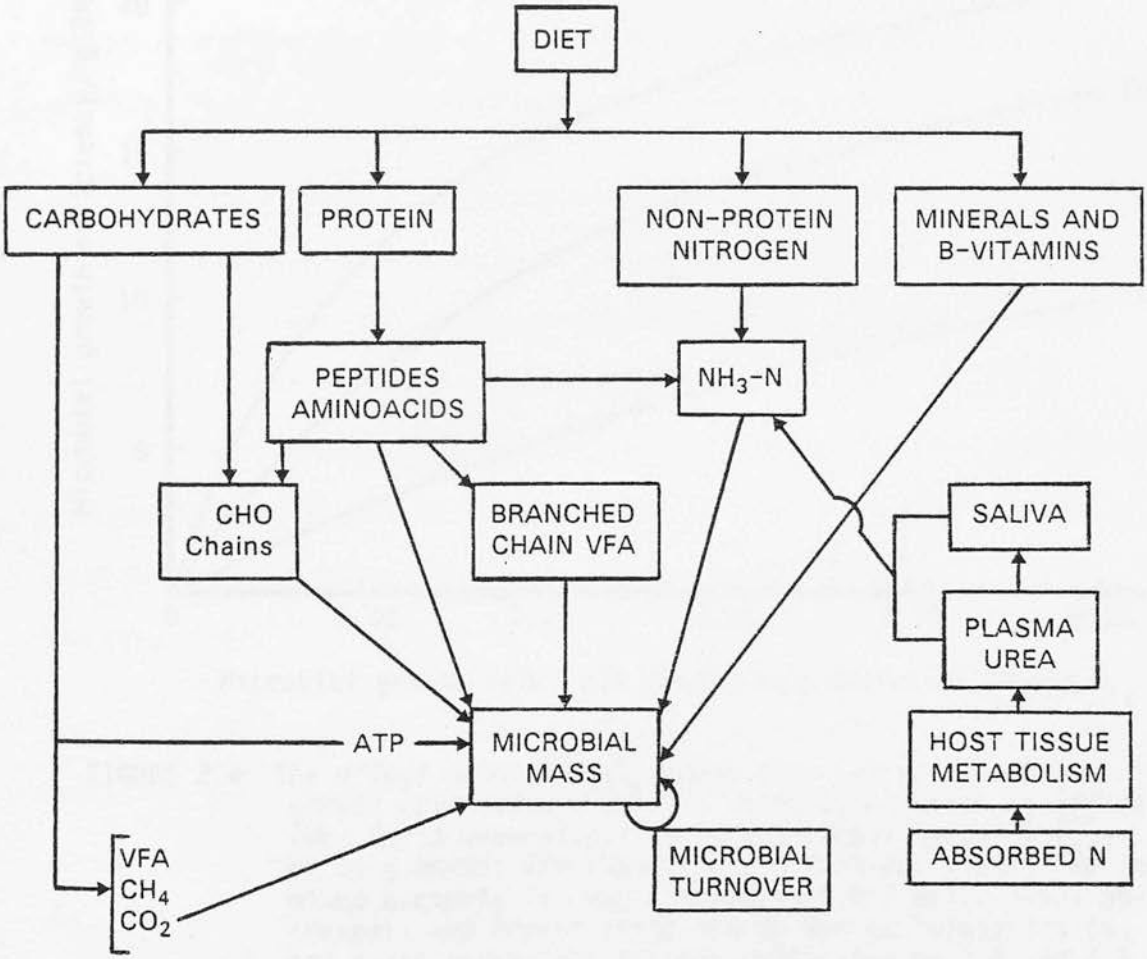

FIGURE 2.3 The sources of nutrients for microbial growth and activity in the rumen.

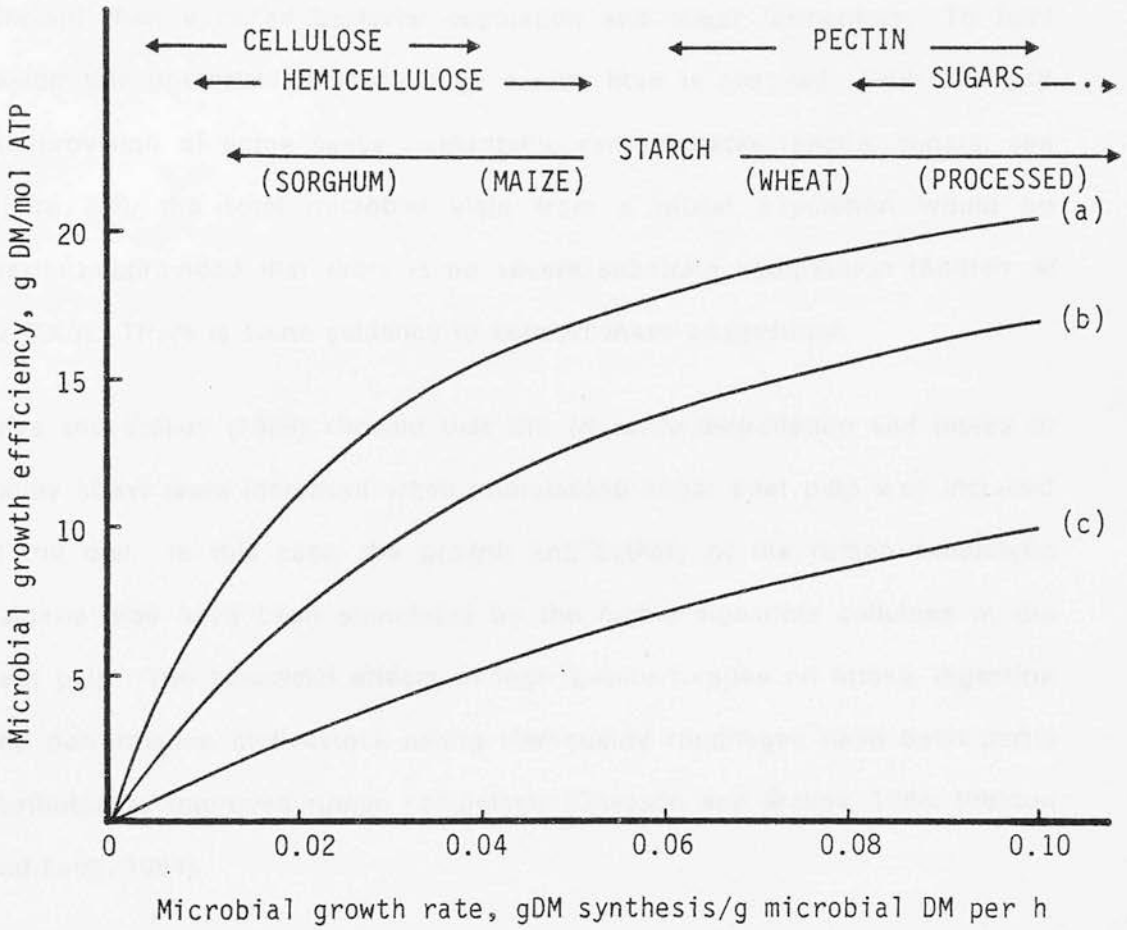

FIGURE 2.4 The effect of microbial growth rate (μ) on microbial growth efficiency (Y^{ATP}) at three maintenance co efficients (Me) for a theoretical maximum microbial yield (Y^{ATP}_{max}) of 26 g DM/mol ATP (Harrison and McAllan, 1980). Me for mixed bacteria (a) was assumed as 0.002 moles ATP/g DM/h (Hespell and Bryant 1979) and Me for cellulolytics (b) and sugar fermenters (c) was calculated as 0.5 and 1.5 of the mean value for mixed bacteria (Sniffen et al, 1983). Where:

$$\frac{1}{Y^{ATP}} = \frac{1}{Y^{ATP}_{max}} + \frac{Me}{\mu}$$

(Adapted from Sniffen et al, 1983)

(sugar fermenters). The growth rates likely to be sustained by various carbohydrates (Sniffen *et al*, 1983) are also shown in Figure 2.4. At the low growth rates expected with highly-fibrous substrates, cellulolytics are more efficient than a mixed bacterial population and sugar fermenters. To fully exploit this potential efficiency, high-quality fibre is required. With this and the provision of some easily fermentable carbohydrates (pectin, sugars, see Figure 2.4), the total microbial yield from a mixed population would be maximized,provided that there is no severe substrate competition (Sniffen *et al*, 1983). There is some evidence to support these suggestions.

Silva and Ørskov (1985) showed that the *in sacco* degradation and intake of barley straw were increased when unmolassed sugar beet pulp was included in the diet. In this case, the growth and activity of the rumen cellulolytic bacteria may have been stimulated by the highly-digestible cellulose in the beet pulp. The beneficial effects of high-quality forages on intake, digestion and performance in livestock eating low-quality roughages have been partly attributed to improved rumen cellulolysis (Chesson and Ørskov, 1984; Preston and Leng, 1984).

The complementary effects of carbohydrate sources on microbial yields in the rumen are clearly illustrated in the results reviewed by ARC (1984). Microbial nitrogen yields (g per kg OM apparently digested in the rumen) were higher for diets with roughage:concentrate ratios of 0.7:0.3 to 0.3:0.7 than for diets outside these ranges. Moreover, ARC (1984) showed that grass and legume forages sustained the highest microbial yields in the rumen when compared to the mean of most other feeds fed to sheep. Except at very late maturity, grass and legume forages contain high-quality fibre (NDF digestibility: 0.6-0.8 for grasses and 0.5-0.7 for legumes; Minson, 1982; Van Soest, 1982). The

levels of soluble sugars and starch in these forages are not sufficiently high to impair fibre digestion in the rumen nor to induce severe substrate competition. Legumes contain appreciable levels of pectin, a soluble carbohydrate which does not seem to impair fibre digestion (Van Soest, 1982) and sustains high microbial growth rates (Figure 2.4).

2.4.3.2.2 Nitrogen

Of the predominant fibre-digesting bacteria, *Ruminococci* and *B. succinogenes* require ammonia as the main source of nitrogen. They also require preformed peptides and aminoacids for maximum growth. *B. fibrisolvens* grows satisfactorily when supplied with either ammonia or mixtures of aminoacids (Bryant, 1973). Assuming a continuous and commensurate supply of nutrients, the protein requirements of rumen bacteria (RDP) in sheep and cattle fed high-roughage diets have been estimated as about 8 g RDP per MJ ME (ARC, 1984). Although the level of ammonia in the rumen fluid results from the balance of its release from feeds and other sources and its uptake by microorganisms, it seems to provide a good indication of the adequacy of nitrogen supply. The minimum NH_3-N required for maximal microbial protein synthesis *in vitro* is 60-80 mg per litre. *In vivo* it varies widely from 70 to 200 mg per litre, probably due to differences in diets and outflow rates of rumen fluids (Buttery, 1977).

The nitrogen incorporated into the microbial mass comes from various sources in the rumen (Figure 2.3). With diets containing over 640 g of low-quality roughages per kg of total diet, 0.53-0.77 of the microbial-N was derived from ruminal NH_3-N; the remainder was directly derived from dietary, endogenous and microbial protein (Al-Rabbat and Heaney, 1978b; Mathison and Milligan, 1971; Nolan and MacRae, 1976; Nolan and Stachiw, 1979; Pilgrim, Gray, Weller

and Belling, 1970). These protein sources seem to fulfil the microbial requirements for preformed peptides and aminoacids in most practical diets (ARC, 1984; Kellaway and Leibholz, 1983). Therefore, ARC (1984) suggested that there is little advantage from the use of protein instead of NPN to supply nitrogen for the rumen microbes, appart from the possible benefit of the slower release of nitrogen from protein. Feeding roughages and N-supplements at 1-2 hourly intervals Kropp, Johnson, Males and Owens (1977) and Petersen, Clanton and Britton (1985) found nonsignificant effects of replacing urea-N with protein-N on microbial protein synthesis in the rumen. When both a low-quality bermuda grass and the nitrogen supplements were fed twice daily, Amos and Evans (1976) obtained increased microbial yields with sunflower meal but not with urea, compared to grass alone. With once a day feeding of corn crop residues, Hefner, Berger and Fahey (1985) found that fibre digestion in the rumen tended to be higher in lambs receiving natural protein supplements (soya bean meal, corn liquour, corn gluten meal) than in lambs supplemented with urea plus branched-chain fatty acids. Therefore, slow release of nitrogen, and probably other nutrients, from protein sources can be advantageous when feed is offered in large and infrequent meals. Frequent feeding (or dosing) and slow releasing of urea and/or energy from synthetic compounds improve roughage intake, N-retention and microbial protein synthesis (Meggison, McMeniman and Amstrong, 1979; Romero, Siebert and Murray, 1976; Smith, 1979; Umunna, 1982).

Bacterial fermentation of leucine, valine and isoleucine in the rumen produces isovaleric, isobutyric and 2-methylbutyric acids, respectively (Hungate, 1975). These acids are essential for maximum growth of most fibre-digesting bacteria (Bryant, 1973). Small additions of branched chain VFA (isobutyric, isovaleric, n-valeric) to high-roughage rations containing urea have resulted in increased

roughage intake, N-balance and microbial protein synthesis (Hemsley and Moir, 1963; Umunna, Klopfenstein and Woods, 1975). With diets containing more fermentable roughage and less rumen degradable protein, the acids become more important. Feeding a mixture of these acids to high-yielding dairy cows led to increased OM digestibility, microbial yield and milk production (Papas, Ames, Cook, Sniffen, Polan and Chase, 1983; Robinson, 1983; (Cited by Sniffen *et al*, 1983)).

2.4.3.2.3 Minerals and vitamins

Macrominerals,trace elements and B-vitamins are either stimulatory or essential for growth of fibre-digesting bacteria. This subject has been discussed in several reviews (Bryant, 1973; Demeyer, 1981; Durand and Kawashima, 1980; Harrison and McAllan, 1980). Recommended mineral dietary concentrations to meet the requirements of rumen bacteria and some classes of livestock offered low quality roughage-based diets are given in Table 2.1. Apart from Mn, Fe and Co, it seems that the dietary allowances for ruminants fed straw-based diets meet the requirements of the rumen microbes. The levels of Ca and Na in the rumen fluid are normally sufficient for the microbes (Durand and Kawashima, 1980). The sulphur requirements of the microbes are well established as a degradable N/degradable S ratio of 14:1 (ARC, 1980).

Mineral supplements containing P and most trace elements are required when cereal straws are fed to ruminants (See Table 2.1). These supplements are usually formulated by considering the suitability of the mineral sources for the requirements of the host. The needs of the rumen bacteria in this respect are seldom taken into account. Thomsen, Moller and Vibe (1978) suggested the use of a slow-releasing mineral mixture (chelated minerals) for maximum digestion of straw-based diets. However, they did not provide evidence of the

TABLE 2.1 Recommended mineral dietary composition for rumen microbes and some classes of ruminant livestock and average mineral composition of cereal straws

	P	Mg	K	Fe	Mn	Zn	Co	Cu
	g/kg DM			mg/kg DM				
Rumen microbes[1]	1.7	0.8	5	120	120	50	0.5–1.0	5–1
Ruminant livestock[2]	2.8–6.7	1.4–1.7	5–8	30–40	40	40	0.11	6–1
Straw composition[3]	1.3	1.1	7.8	–	28	23	–	5

1. From Durand and Kawashima (1980). Taking OMD = 0.46 and Ash = 77 g/kg DM for straws (Tables 2.4 and 2.5).

2. ARC (1980); MAFF-DAFS-DANI-UKASIA-BVA (1983). DM intake for coarse diets with ME/GE = 0.4 (Tables 2.4 and 2.5)
Growing cattle (200–300 kg LW, 500 g LWG/day); pregnant cows (500 kg LW, last 2 months); pregnant ewes (70 kg LW, last 2 months.

3. From Table 2.4

superiority of these mixtures over conventional mineral supplements.

Most fibre-digesting bacteria require one or more of the B-vitamins. Among the vitamins identified to be essential or stimulatory for growth are: biotin, p-aminobenzoic acid, pyridoxine, folic acid, riboflavin, thiamin and cobalamin (Bryant, 1973).

2.5. KINETICS OF FIBRE DIGESTION IN THE RUMEN

The utilisation of fibre in the rumen involves two interacting processes: digestion and passage. Digestion comprises microbial attachment and enzymic action, while passage includes particle size reduction not effected by enzymic action, and escape from the rumen (Mertens and Ely, 1982). Complex relationships among host, microbes and plant components affect these processes. Microbial and plant factors were discussed in previous sections. For a detailed account on the importance of host factors, see Mertens and Ely (1979,1982).

Several comprehensive models of ruminant digestion have been developed to evaluate forage utilisation (Baldwin, Koong and Ulyatt, 1977; Black, Beever, Faichney, Howarth and Graham, 1981; Beever, Black and Faichney, 1981; Mertens and Ely, 1979; Poppi, Minson and Ternouth, 1981c). Regarding digestion of cell walls, all models are based on a simple principle: fibre can be divided into potentially digestible and indigestible fractions; the former can disappear from the rumen by digestion and passage whereas the latter can disappear only by passage (Waldo, Smith and Cox, 1972). However, there are several reasons which make it necessary to expand this principle into more complex models. Firstly, fibre is comprised of various fractions with different rates of digestion. Secondly, passage of fibre may be affected by many interrelated factors such as particle size distribution, particle breakdown within

the rumen, level of intake and physico-chemical characteristics of the fibre. An integrated view of the flow of fibre through the rumen is given in Figure 2.5 for a model with two pools of different particle size. In the following sections fibre digestion and passage are discussed in more detail. Hereafter, the idea of digesta "pools" is referred to in the context of models of rumen digestion. It is recognized that such "pools" do not strictly exist in reality but the idea is useful to understand the processes of digestion and passage ocurring in the alimentary tract.

2.5.1. Digestion of fibre in the rumen

In vivo and *in sacco* fermentation-time curves for fibrous feeds follow the general patterns shown in Figure 2.6, depending on whether cumulative disappearance (Figure 2.6a) or the substrate residue at each incubation time (Figure 2.6b) are expressed on the Y-axis. Mathematical models describing these curves yield three parameters with some biological meaning. In both curves, lag time (T) refers to a period between incubation time zero and the onset of fermentation. During this phase, digestion is either zero or occurs at a very slow rate not detectable with existing techniques (Mertens, 1977). The asymptote of the curve represents the potentially digestible fraction of the substrate (D) (Figure 2.6a) or the indigestible fraction (I) (Figure 2.6b). The third parameter (not illustrated in the figures but included in the model equations given below) is the digestion rate (k) which indicates the amount of substrate fermented per unit time.

The model in Figure 2.6a can be described by the following equations for cumulative disappearance before and after the lag time (McDonald,1981) :

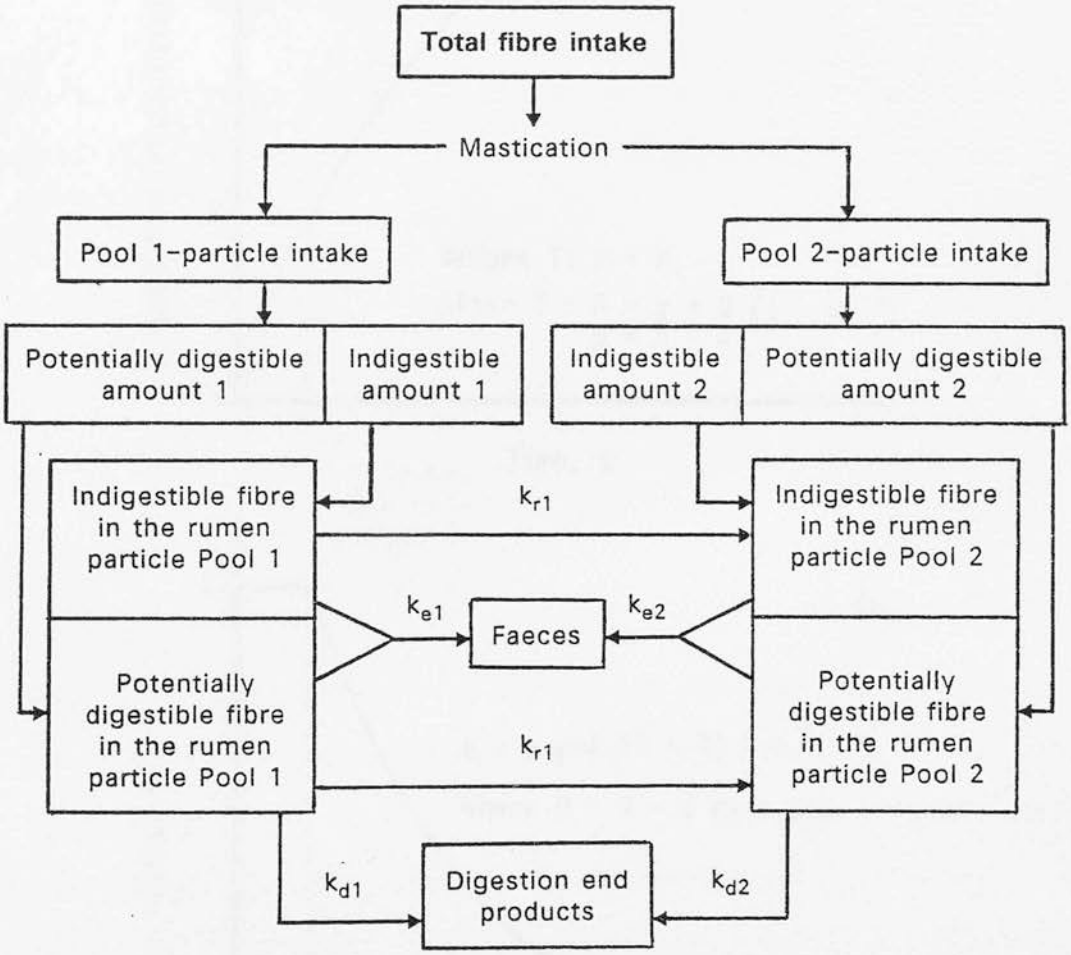

FIGURE 2.5 Diagram illustrating the flow of fibre in the rumen for a two-particle size pool model. k_d = rate of digestion; k_r = rate of particle size reduction; k_e = rate of escape from the rumen. Mean particle size in Pool 1 is larger than in Pool 2. Particles in Pool 2 can undergo further reduction to a third or subsequent pools depending on the model. (Adapted from Poppi *et al*, 1981c).

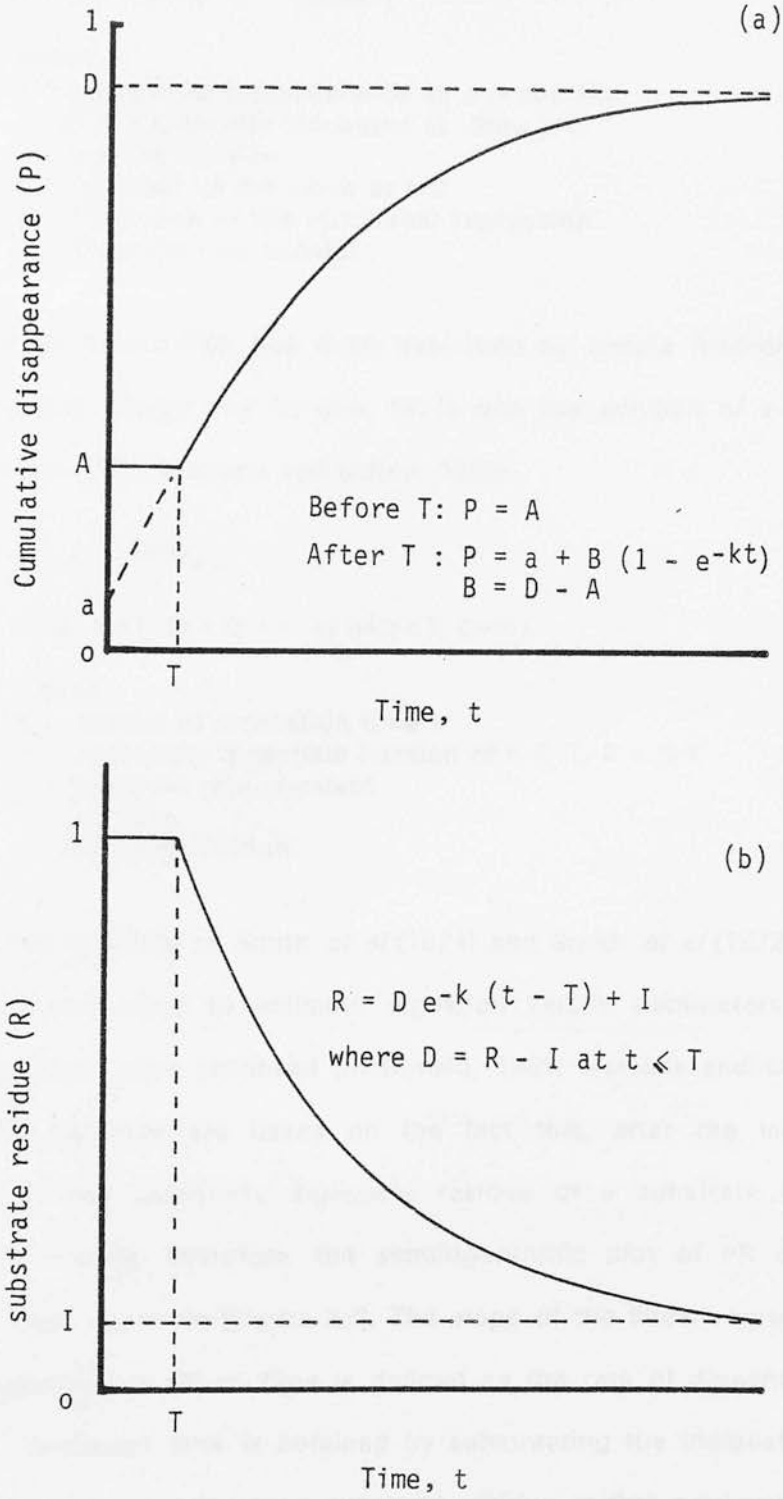

FIGURE 2.6 Relationship between fermentation time and: (a) cumulative substrate disappearance (McDonald, 1981) and (b) substrate residue (Mertens and Loften, 1980) for fibrous feeds incubated *in vitro* and *in sacco*.

$P = A$; before T (Equation 1)

$P = a + B\,(1-e^{-kt})$; after T (Equation 2)

where;
P = cumulative disappearance as a proportion of the substrate incubated at time t=0
A = soluble fraction
a = intercept of the curve at t=0
B = parameter of the non-linear regression
k = digestion rate constant

The model in Figure 2.6b has been described by simple first-order kinetics (Smith, Goering, Waldo and Gordon, 1971) with the addition of a discrete lag time (Mertens, 1977; Mertens and Loften, 1980)

$$R = D\,e^{-k(t-T)} + I \qquad \text{(Equation 3)}$$

when $t>T$; $R = D + I$ at $0<t<T$, $D+I=1$

where;
R = residue at incubation time t
D = potentially digestible fraction at $t < T$, $D = R-I$
k = digestion rate constant
T = lag time
I = insoluble fraction

Although the methods of Smith *et al* (1971) and Smith *et al* (1972) have been most commonly used to estimate digestion kinetic parameters, alternative techniques have been proposed (McDonald, 1981; Mertens and Loften, 1980). The earlier methods are based on the fact that, after the lag time, the digestion of the potentially digestible residue of a substrate (PR) follows first-order kinetics. Therefore, the semilogarithmic plot of PR against time shows a linear decrease (Figure 2.7). The slope of the linear regression of the natural logarithm of PR on time is defined as the rate of digestion. PR for a particular incubation time is obtained by substracting the indigestible fraction of a substrate (I) from the total substrate residue at that particular incubation time. The validity of the first-order kinetic model depends on the incubation time selected to estimate the indigestible fraction (I) (Mertens, 1977).

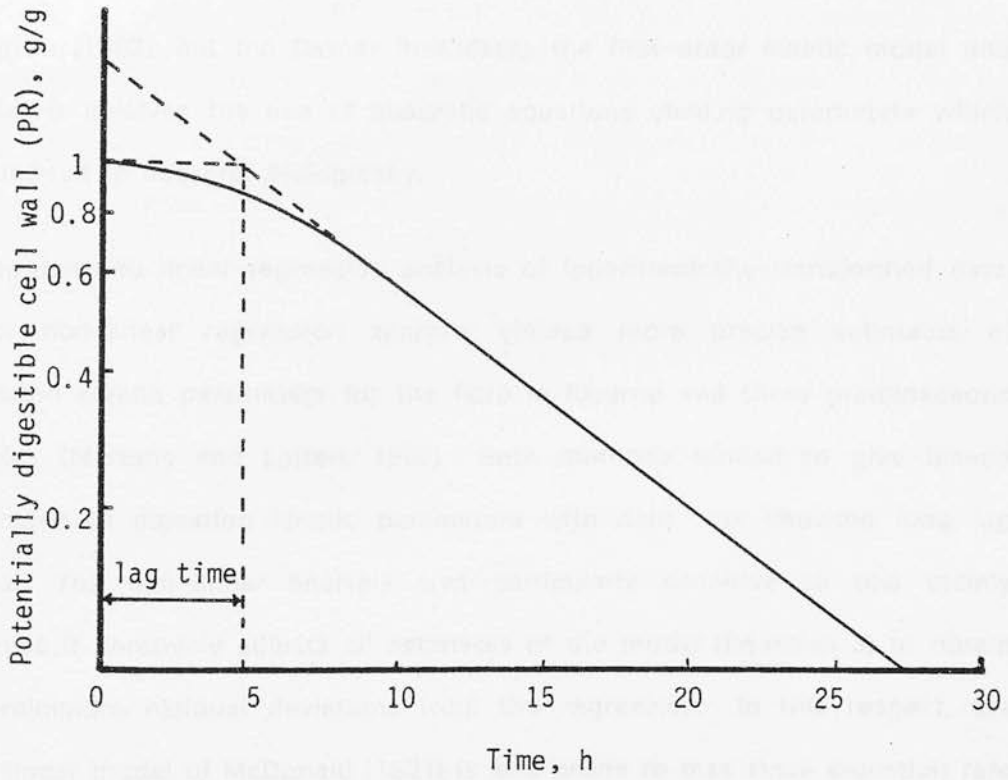

FIGURE 2.7 Semilogarithmic plot of the potentially digestible cell wall residue on incubation time *in vitro* (Mertens, 1977)

Incubation times between 48 h and 96 h have been used, but 72 h is more widely accepted (Belyea, Darcy and Jones, 1979; Miller and Muntifering, 1985; Zorrilla-Rios, Owens, Horn, McNew, 1985). Although digestion of cell walls occurs after 72 h, little fibre is retained in the rumen beyond 72 h and this end point does not invalidate the first-order kinetic model (Mertens, 1977; Van Soest, 1982). Alternative methods of estimating the indigestible fraction (I) have been proposed by Mertens and Van Soest (1972) and Dryden and Kempton (1983), but the former invalidates the first-order kinetic model and the latter involves the use of quadratic equations yielding parameters which are difficult to interpret biologically.

As opposed to linear regression analysis of logarithmically-transformed data, direct non-linear regression analysis yielded more precise estimates of digestion kinetic parameters for the fibre in lucerne and three graminaceous species (Mertens and Loften, 1980). Both methods tended to give biased estimates of digestion kinetic parameters with data sets showing long lag times. The non-linear analysis was particularly sensitive to this mainly because it iteratively adjusts all estimates of the model (Equation 3) to obtain the minimum residual deviations from the regression. In this respect, the non-linear model of McDonald (1981) is less prone to bias since digestion rate (k) and the potentially digestible fraction are estimated with an equation (Equation 2) different from that for lag time; *i.e.*:

$$T = 1/k \ln [B/ (a+B)- A] \qquad \text{(Equation 4)}$$

where all variables are defined as in Equation 2

However, a previous visual appraisal of the fermentation data is required to estimate lag time roughly, and exclude values before this time, when fitting

Equation 2 in the model of McDonald (1981). The estimation of lag time according to this model is influenced by the value of the soluble fraction (A) (Equation 4). With components such as protein, this may be an additional source of error since there is not full agreement on the best method to determine the truly soluble fraction (ARC, 1984). However, this is not the case for fibre in forages where the soluble fraction is zero (Van Soest, 1985).

Estimates of kinetic parameters of digestion are useful to understand their influence on intake and *in vivo* digestibilility of forages. These could be be predicted through dynamic models based on results from *in vitro* or *in sacco* incubations. Moreover, there are possibilities of using digestion kinetic parameters to formulate rations considering their intake potential, in addition to their chemical composition (Varga and Hoover, 1983). Mertens and Ely (1982) compared the observed intakes and *in vivo* digestibilities of 166 forages by sheep with predicted values from the model of Mertens and Ely (1979). *In vitro* estimates of digestion kinetic parameters and a constant rate of passage were used to develop the model. About 0.5 of the variation in digestibility among forages could be accounted for by differences in the kinetic parameters determined. Generally, better predictions were obtained for DM digestibility than for DM intake and, in both cases, the model underpredicted values for low-quality roughages. This may have been partly due to a higher digestibility and intake in the diet selected by sheep as compared to the sample prepared for *in vitro* fermentation. In addition, the model did not adjust for differences in rate of passage which is one of the main factors affecting the behaviour of comprehensive models to predict the nutritive value of low-quality roughages (Baldwin, Koong and Ulyatt, 1977).

Compared to potential degradability, digestion rate seems to have a minor

influence on the control of forage intake and digestibility. The model simulations of Mertens and Ely (1979) showed that the maximum intake of digestible DM was affected to a greater extent by the rate of passage of digesta through the digestive tract and the indigestible fibre fraction (1-potential digestibility) than by the rate of digestion. Mertens (1973) (Cited by Van Soest, 1982) reported a low correlation (r=-0.1) between intake and digestion rate of cell walls for 187 forages. Coombe, Dinius and Wheeler (1979) did not find any significant relationship between digestion rate and intake of untreated and NaOH-treated straws by steers. A good relationship between the intake of four hays by sheep and their potential degradability *in sacco* was shown graphically by Hovell, Ngambi, Barker and Kyle (1986) (no correlation coefficient was reported). Chenost, Grenet, Demarquilly and Jarrige (1970) reported a correlation of r=0.74 between the 24-h *in sacco* degradation and intake of 35 grass hays.

The specific effects of lag time on *in vivo* digestibility and intake of forages have not been studied. However, there is some evidence to suggest that lag time may be inversely correlated with intake and digestibility of forages. Mertens and Loften (1980) showed that the lag time for *in vitro* digestion of forage fibre was prolonged with increasing levels of starch in the fermentor. Similarly, Miller and Muntifering (1985) found that the lag time for the *in sacco* degradation of forage fibre increased as the level of maize in the diet of steers increased. Increasing levels of supplementation with starchy feeds are associated with decreasing forage intake and fibre digestibility (ARC, 1980; Chimwano *et al*, 1977). Moreover, the increased intakes and digestibilities of two cereal straws and a low-quality hay by steers due to alkali treatment were associated with decreased lag times (Thiago, Kellaway and Leibholz, 1979).

2.5.2. Passage of fibre through the rumen

Fibre disappearance from the rumen plays a crucial rôle in the control of intake of roughages (Van Soest, 1982). Moreover, the model simulations of Baldwin *et al* (1977) indicated that passage of digesta and particle size reduction in the ruminal digestion process may be critical. The rate of passage of digesta through the gastrointestinal tract is influenced by several factors, including: animal species, level of intake, type and physical form of the diet, physiological state, frequency of feeding and ambient temperature (Warner, 1981). With roughages fed in the long form, the rate of comminution of large particles to attain proper physical characteristics to leave the rumen has been thought to be a critically restrictive determinant (Balch and Campling, 1962; Chesson and Ørskov, 1984; Welch, 1982). This has been clearly identified as a subject requiring more extensive research (Baldwin *et al*, 1977; Mertens and Ely, 1979).

For feed particles to leave the rumen they need not only to be broken down but also sorted to the bottom surface of the rumen and expelled through the reticulo-omasal orifice. Only those particles in the cranial and ventral sacs of the rumen are propelled through the reticulo-omasal orifice (McBride, Milligan and Turner, 1983,1984). Digesta particles reach these sites mostly because of their size and density and therefore, the processes of size reduction and sorting of particles seem to be determinant in the control of digesta transfer to the omasum.

2.5.2.1. Particle size reduction

Three main processes are involved in the size reduction of feed particles: a) chewing during eating and ruminating; b) microbial fermentation and; c) mechanical detrition by rumen contractions. Chewing during rumination plays

a major rôle (Milligan, Kennedy and Khristopherson, 1980; Murphy and Nicoletti, 1984; Poppi *et al*, 1981c; Reid, John, Ulyatt, Waghorn and Milligan, 1979; Welch, 1982). Therefore, those factors controlling rumination directly affect the rate of particle size reduction in the rumen.

2.5.2.1.1 Rumination and size reduction of feed particles

Rumination is closely related to the physical and chemical characteristics of the diet. As the long fibre fraction is largely responsible for rumination activity (Welch,1982), rumination time is longer when roughages are fed long rather than ground and pelleted (Dixon and Milligan, 1985; Kennedy, 1985). Rumination is also a function of the concentration of soluble matter and fibre in the forage. The soluble fraction seems to affect rumination through changes in rumen pH and osmotic pressure: low pH and high osmotic pressure may impair rumination (Welch,1982). Forages with high fibre concentration are associated with long rumination times and high rumination rates (g cell wall per min.) (Welch and Smith, 1969; Van Soest, 1982). Since total rumination time does not exceed 10–12 h per day, regardless of species and body size within species, rumination efficiency (g cell wall per min) becomes determinant for the intake of long roughages (Welch, 1982; Weston and Kennedy, 1984). Sheep have a lower rumination efficiency than cattle while mature cattle and sheep ruminate more g cell wall per min than their younger counterparts (Welch, 1982).

2.5.2.1.2. Methods of particle size analysis in feed, digesta and faeces

It is difficult to draw definitive conclusions from reported results on quantitative estimates of particle breakdown in the rumen and its relation to forage intake. The measurement of rates of particle size reduction is cumbersome and most of the techniques used in the experiments reported are

not entirely satisfactory. This is particularly so for the methods of size fractionating of feed, digesta and faeces; *e.g.* dry and wet sieving of samples on a column of sieves of known aperture size. Although dry sieving is associated with lower variation of replicate samples than is wet sieving, it is less generally accepted as an appropriate procedure for digesta and faecal samples (Allen, Robertson and Van Soest, 1984). Dry sieving tends to separate particles according to their minimum dimension as they freely bounce around on the sieves and requires drying and subsequent breaking of wet samples (Uden and Van Soest, 1982). The former would not occur in the rumen while the latter may alter the original particle size distribution in the sample. The dry sieving technique of extracted cell walls (Smith and Waldo, 1969), used in the experiments of Smith and co-workers (Smith, Waldo, Moore, Leffel and Van Soest, 1967; Smith, Weinland, Waldo and Leffel, 1983) seems less widely accepted (See Kennedy, 1984).

Although wet sieving seems to be a more favoured technique there are major differences in the methods reported regarding sample size, sample size: sieve area ratio, number of sieves and their aperture size, sieving time and shaking and flushing procedures (Table 2.1A). A comprehensive study of the factors affecting the accuracy of the wet sieving technique for particle separation in feed, digesta and faeces, has not been carried out but more consistent results could be obtained when the following conditions are fulfilled: high sieve area: sample size ratio to prevent formation of a compact mat of particles; long sieving time; strong shaking and copious flushing of water through the sieves (Jones and Moseley, 1977). Moreover, there is a lack of uniformity among research groups on the method of expressing results from experiments on particle fractionation. Direct measurements (*e.g.* using a graticule and a microscope to measure the length of particles smeared on a slide) or

theoretical calculations of particle dimensions are preferred (McLeod, Kennedy and Minson, 1984; Vaage, Shelford and Moseley, 1984).

2.5.2.1.3 Estimates of rates of size reduction of feed particles in the rumen

There are very few reports on rates of particle size reduction in the rumen, with considerable variation among the values estimated (Table 2.2). Besides the inadequacies of the techniques discussed previously, one or more of the following factors may contribute to this variation: definition of particle size reduction rate; selection of the particle sizes to estimate particle size reduction rates; type of diet and animal species. The influence of the last two factors through their effect on rumination has been discussed in a previous section. (Section 2.5.2.1.a). The remaining factors are discussed below.

a. Definition of particle size reduction rate

Feed particles can disappear from a particular particle-size pool in the rumen by digestion (k_d), escape or passage (k_e) and reduction to smaller particles (k_r) (Figure 2.5) (Poppi *et al*, 1981c; Van Soest, 1982). The total rate of disappearance of feed particles from a given pool (k_t) is:

$$k_t = k_d + k_e + k_r \qquad \text{(Equation 5)}$$

There are clear differences in what several authors have reported as rate of particle size reduction. Poppi *et al* (1981c) assumed that the rate of particle size reduction (k_r) was equivalent to the rate of disappearance (k_t) of long particles (retained on a 1.18-mm screen) from the rumen. Evans, Pearce, Burnett and Pillinger (1973) also reported total rates of disappearance (k_t) as rates of breakdown (k_r). These assumptions may not always be correct. With low- to medium-quality forages, the rates of escape (k_e) of particles retained on sieves with aperture size greater than 1.0 mm vary widely: 0.001 to 0.048

TABLE 2.2 Estimated rates of disapearance of faed particles from different particle-size pools in the rumen of sheep and cattle eating forages.

Rate reported, per h				Range of particle size, mm			Type of diet	Frequency of feeding	Estimation procedure	Reference
Definition[1]	Mean	Range	n[2]	Lower	Upper	Species				
k_t	0.040	0.038–0.041	3	>0.60	<0.60	Cattle	Long hay	1 x day	Particle size distribution in rumen samples taken at intervals post-feeding. k_t=slope of linear regression of proportion of large particles and time	Evans *et al* (1973)
	0.044	0.011–0.060	9	>2.40	<2.40					
k_r+k_d	0.156			>1.60	1.60–1.00	Cattle	Grass pastures	Grazing	Concentration of rare-earth markers in rumen and faecal samples taken at intervals post-dosing with marked particles of known mean size	Lascano (1979) (cited by Van Soest, 1982)
	0.074			1.60–1.00	1.00–0.80					
	0.158			1.00–0.80	0.80–0.50					
	0.058			0.80–0.50	0.50–0.30					
	0.043			0.50–0.30	0.30–0.16					
	0.025			0.30–0.16	<0.16					
k_t	0.055	0.041–0.077	8	>1.18	<1.18	Cattle	Chopped dried grasses 2.5 cm	24 x day	Particle size distribution in rumen samples taken once after a 24-h feeding cycle. k_t=inverse of mean retention time of particles >1.18 mm.	Poppi *et al* (1981c)
	0.088	0.077–0.105	8	>1.18	<1.18	Sheep				
k_t	0.044		4	>3.2	<3.2	Cattle	Long hay	12 x day	As Poppi *et al* (1981c)	Dixon and Milligan (1985)

1. $k_t=k_d+k_e+k_r$; where for a particular particle-size pool; k_t=total rate of disapearance; k_d=rate of digestion; k_r=rate of particle size reduction (See text).
2. Number of individual observations or means reported.

per h (Table 2.3); a much wider range than that suggested by Poppi *et al* (1981c) for large particles retained on a 1.18-mm screen (0.002-0.003 per h). Considering the range of k_e values given above (From Table 2.3) and the rates of digestion (k_d) suggested by Poppi *et al* (1981c) for large particles (0.012-0.018 per h), it can be calculated that on average $k_e + k_d$ can represent 0.74 of the average k_t from the data of Dixon and Milligan (1985); Evans *et al* (1973) and Poppi *et al* (1981c) (Table 2.2). Thus, the rate of particle size reduction (k_r) may not always be a major contributor to the total rate of disappearance (k_t). The rates of particle size reduction reported by Lascano (1979) (Cited by Van Soest, 1982) (Table 2.2) are presumably the sum of $k_d + k_r$ since k_e values are reported separately and the rare-earth elements used by this author to mark feed particles were not likely to impair their rate of digestion (k_d) (Hartnell and Salter, 1979). More research is required to obtain more precise values for rates of particle size reduction (k_r), which are more relevant to the indigestible forage fibre fractions: a primary restricting factor for the intake of roughages (Mertens and Ely, 1979).

b. Selection of the particle sizes to determine rates of particle size reduction of feeds in the rumen

The rates of size reduction for feed particles in the rumen vary quite considerably depending on the mean particle size before and after comminution (Table 2.2). Therefore, the influence of particle size reduction on the rate of passage and intake of forages may depend on the particle size limits selected to divide the reticulo-rumen digesta into two or more pools. In fact, there is disagreement on whether, two, three or more particle pools should be considered (Lascano, 1979 (Cited by Van Soest, 1982); Matis, 1972; Mertens and Ely, 1979; Poppi, Norton, Minson and Hendricksen, 1980; Poppi,

TABLE 2.3 Estimated rates of escape of feed particles of different size from the rumen of sheep and cattle eating forages.

Rate of escape(k_e), per h			Particle size, mm^2	Species	Type of of diet	Frequency of feeding	Estimation procedure	Reference
Mean	Range	n[1]						
0.045		1	>2.4	Sheep	Long hay	2 x day	Concentration of Cr in faecal samples taken at intervals after dosing with Cr-mordanted fibre particles of known size. K_e=slope of the descending linear portion of the plot of log concentration versus time (Grovum and Williams, 1973)	Uden and Van Soest (1982)
0.056		1	1.20-0.60					
0.043		1	2.4-1.2					
0.050		1	0.6-0.3					
0.012		4	6.80-4.00	Cattle	Long hay	12 x day	Particle size distribution in rumen and faecal samples taken once after a 24-hr feeding cycle. k_e=ratio flow of a particle-size DM from the rumen (estimated from faecal flows): rumen DM pool size for that group	Dixon and Milligan (1985)
0.026		4	4.00-3.20					
0.040		4	3.20-2.00					
0.048		4	2.00-1.00					
0.021	0.020-0.023	4	>1.00	Cattle	Pastures	Grazing	As Lascano (1979)[3] (Table 2.2)	Ellis, Lascano and Matis (1979)
0.024	0.021-0.026	5	1.00-0.42					
0.001			>1.2	Sheep	Chopped straw	8 x day	Acid detergent fibre determination for each particle size pool in rumen digesta collected after slaughtering; k_e=ratio of ADF leaving the rumen: ADF present in the rumen for each particle pool	Weston and Cantle (1984)
0.003			1.2-0.60					
0.018			0.60-0.30					
0.049			<0.30					
0.007			1.60-1.00	Cattle	Pastures	Grazing	See Table 2.2	Lascano (1979)[3]
0.016			1.00-0.80					
0.015			0.80-0.50					
0.019			0.50-0.30					
0.026			0.30-0.16					
0.037			<0.16					

1. Number of individual observations or means reported.
2. Aperture size of sieves where particles are retained.
3. Cited by Van Soest (1982).

Hendricksen and Minson, 1985).

Basically the fibrous material in the reticulo-rumen can be divided into two pools: large particles, which cannot escape the rumen without being comminuted and small particles, which can leave the rumen more easily (Baldwin, Koong, Ulyatt and Smith, 1976; Hungate, 1966; Poppi *et al*, 1980; Poppi *et al*, 1985; Reid, Ulyatt and Munro 1977; Ulyatt, Baldwin and Koong,1976). With this approach, two important issues should be considered: a) the critical size for particles leaving the rumen and; b) the homogeneity of size, breakdown and escape of particles within each pool.

Critical size for feed particles leaving the rumen

Although the critical size for feed particles leaving the rumen is not clearly defined, this seems to be the mean size for particles retained on sieves with aperture size of 1.00-1.18 mm. Several workers have shown that very few particles in the post-ruminal digesta and in the faeces of sheep and cattle are retained on sieves with aperture sizes of 1.00-1.18 mm (Pearce, 1967; Poppi *et al*, 1985; Poppi *et al*, 1980; Reid *et al*, 1977; Troelsen and Campbell, 1968; Ulyatt, 1983). It has been suggested that ruminant species, level of intake and type of feed influence this critical size (Chesson and Ørskov, 1984; Troelsen and Campbell, 1968; Van Soest, 1966;1982). However, there is no fully convincing evidence to support some of these suggestions. Poppi *et al* (1980) found a tendency for higher mean particle size in post-ruminal digesta (Modulus of fineness of the American Society of Agricultural Engineers (ASAE),1967) to be associated with higher intakes of legumes and grasses by sheep. However, this was due to changes in the particle size distribution on the sieves with apertures smaller than 1.18 mm, with no variation in the critical size for particles leaving the rumen. This may have also happened in

the experiments of Van Soest (1966), where the mean faecal particle size (Geometric mean of Waldo, Smith, Cox, Weinland and Lucas, 1971) increased with the level of intake of hay and silage by lactating cows. Moreover, Bae, Welch and Smith (1981) reported that particle size distributions in faeces did not differ with the level of hay intake in Holstein cows. Troelsen and Campbell (1968) reported a decreasing proportion of omasal digesta particles retained on a 0.25-mm sieve associated with decreasing intakes of several grasses and legumes by sheep, but information on only one particle size could be misleading. For similar reasons, it could be concluded that the critical size for particles passing to the omasum may not be affected by forage species and maturity (Poppi *et al*, 1980; Poppi *et al*, 1985; Troelsen and Campbell,1968)

The mean particle size in sheep faeces is smaller than in cattle faeces (Poppi *et al*, 1985; Thomas and Campling, 1977; Uden and Van Soest, 1982). In contrast to the differences in mean particle size due to level of intake and forage type and maturity, this can be attributed to a higher proportion of large forage particles in cattle faeces than in sheep faeces (Poppi *et al*, 1985). However, based on the relative resistance of particles to passage from the rumen, Poppi *et al* (1985) suggested that a critical particle size of 1.18 mm can be taken to divide the rumen contents of both cattle and sheep into large and small particle pools.

Size, breakdown and passage of particles within a particular particle-size pool in the rumen digesta

The size, breakdown and passage of particles are heterogeneous within the large-particle pool (retained on 1.00-1.18 mm sieves) and the small particle-pool (passing 1.00-1.18 mm sieves) in the rumen digesta (Dixon and Milligan, 1985; Evans *et al*, 1973; Lascano, 1979 (Cited by Van Soest, 1982);

Pearce, 1967; Poppi *et al*, 1980; Poppi *et al*, 1985; Reid *et al*, 1977; Troelsen and Campbell, 1968; Weston and Cantle, 1984). This may be a disadvantage of the two-pool model, but simpler models are preferable (Poppi *et al*, 1980; Poppi *et al*, 1985) since the analysis and interpretation of multi-pool models is rather complex (Matis, 1972; Poppi *et al*, 1980; Van Soest, 1982).

Mertens and Ely (1979) suggested a model for the rumen with three particle-size pools: a) large, with particles retained on a 2-mm screen; b) medium, with particles passing a 2-mm screen but retained on a 0.5-mm screen and; c) small, with particles passing a 0.5-mm screen. The selection of three particle-size pools was justified by Mertens and Ely (1979) in view of the trimodal distribution of particle sizes in the rumen reported by Ulyatt *et al* (1976) and Evans *et al* (1973) and the most precise description of the excretion of marker in the faeces of ruminants achieved with a tri-subcompartmental mathematical model by Matis (1972). A trimodal distribution of rumen particles was also apparent in other studies (Dixon and Milligan, 1985; Pearce, 1967). However, the best fit of a tri-subcompartmental model to marker excretion in faeces seems to reflect the presence of three mixing compartments in the gastro-intestinal tract (Warner,1981) rather than the existence of three particle-size pools within the rumen. Based on the relative resistance of particles to leaving the rumen, Poppi *et al* (1985) also identified three pools in the rumen of sheep and cattle. However, according to them the quantity of large particles (retained on a 1.18-mm screen) appearing in the faeces and their resistance to flow compared to small particles, did not justify the use of three pools for modelling studies. They also suggested that the behaviour of different particle pools may depend on the aperture size of the sieves selected to define these pools (See Tables 2.2 and 2.3) and that spurious results could be obtained unless a mathematical model to remove this sieve-size bias is

used. This will also apply to multi-pool models of rumen particle size. Lascano (1979) (Cited by Van Soest, 1982) divided the rumen into six pools to develop a model considering rates of ingestion, size reduction and escape for the different particle sizes (Tables 2.2 and 2.3). This approach of a multiple-pool model may reflect more closely the digestion and passage processes in the rumen while the two-pool model may be an oversimplification. However, a systematic study comparing the effect of defining two or more pools on the estimation of particle size reduction rates and their influence on intake and passage has not been reported. With this rather unexplored field of research, it is perhaps more important to refine and standarize the techniques used to obtain consistent results within the frame of simple theoretical models.

2.5.2.2. Sorting of digesta particles

Particle size, density and shape are involved in the sorting of digesta particles within the rumen (Campling and Freer, 1962; Ehle, 1984; Evans *et al*, 1973; Troelsen and Campbell, 1968). There is evidence to suggest that changes in particle density are largely a result of changes in particle size. From the results of Evans *et al* (1973) and Hooper and Welch (1985b) it seems that particle size and density in digesta are inversely correlated. Adsorption of water and ions, which occurs mainly through carboxyl groups in hemicellulose and phenol groups in lignin, leads to an increased density of fibre particles (Hooper and Welch, 1985a,b; Van Soest, 1982). Particle comminution increases surface area and therefore the likelihood of these carboxyl and phenol groups being exposed to the surrounding fluids increases. During microbial fermentation in the rumen, the volume of the structure of plant fibre is not reduced since the digestible fractions extracted from this structure are replaced by gas, water and bacteria. Therefore, the density of the fibre

structure can only be reduced by disintegrating the structure itself through particle size reduction (Van Soest and Robertson, 1976).

Troelsen and Campbell (1968) suggested that the shape of ingested plant particles may be an important factor controlling the stratification of digesta in the rumen and its onward passage. They showed that the omasal digesta particles in sheep eating grasses were generally long and thin while those in sheep eating legumes of similar maturity were short and broad.

Despite their size, density and shape, small particles cannot always reach the ventral portions of the reticulo-rumen with ease. This has been atributed to entrapment of small particles within the fibrous mat in the rumen (Poppi *et al*, 1985; Welch and Smith, 1978).

2.5.2.3. The effect of size reduction of feed particles in the rumen on forage intake

The widely accepted theory of the limiting effect of particle size reduction rate on passage and intake of forages in ruminants (Balch and Campling, 1962) was tested by Poppi *et al* (1981c) through an intake simulation based on a model similar to that shown in Figure 2.5. The results of the model simulations indicated that forage intake and mean retention time of DM in the rumen were affected to a larger extent by the rates of passage and digestion of small particles (passing through a 1.18-mm screen) than by the breakdown of large particles (retained on a 1.18-mm screen). These results were partly attributed to the fact that large particles only contributed about 0.3 of the DM in the rumen, a value similar to that reported by Pearce (1967) for sheep fed coarsely chopped oaten hay but different from the values obtained by Dixon and Milligan (1985) and Kennedy (1985). These workers found that for sheep and cattle consuming long grass and legume hays, the particles retained on a

1.0-mm sieve represented 0.5 to 0.65 of the DM in the rumen. As discussed previously, large differences in the sieving techniques used are likely to be responsible for some of the variation among the results reported in the literature. The results of Poppi *et al* (1981c) are for coarsely chopped forages (2.5 cm long) fed *ad libitum* and at hourly intervals. Moreover, their rates of particle size reduction were estimated as the inverse of the rumen turnover time of DM. With this approach, the rates of particle size reduction, escape and digestion are confounded (Mertens and Ely, 1979). The importance of size reduction of feed particles for intake of long roughages fed in large and infrequent meals is still to be assessed.

Size reduction of feed particles may limit the outflow rate of digesta from the rumen even for particles considered to be small enough to leave this organ without further comminution. Lascano (1979) (Cited by Van Soest, 1982) observed that for forage particles passing a 1-mm sieve, the rates of size reduction in the rumen (k_r) decreased while the rates of escape from the rumen (k_e) increased as the mean particle size decreased (See Tables 2.2 and 2.3). As a result the ratio of the input to the output of particles for a given rumen pool (*i.e.* k_r/k_e) decreased rapidly as the mean particle size of the pool decreased (Figure 2.8).

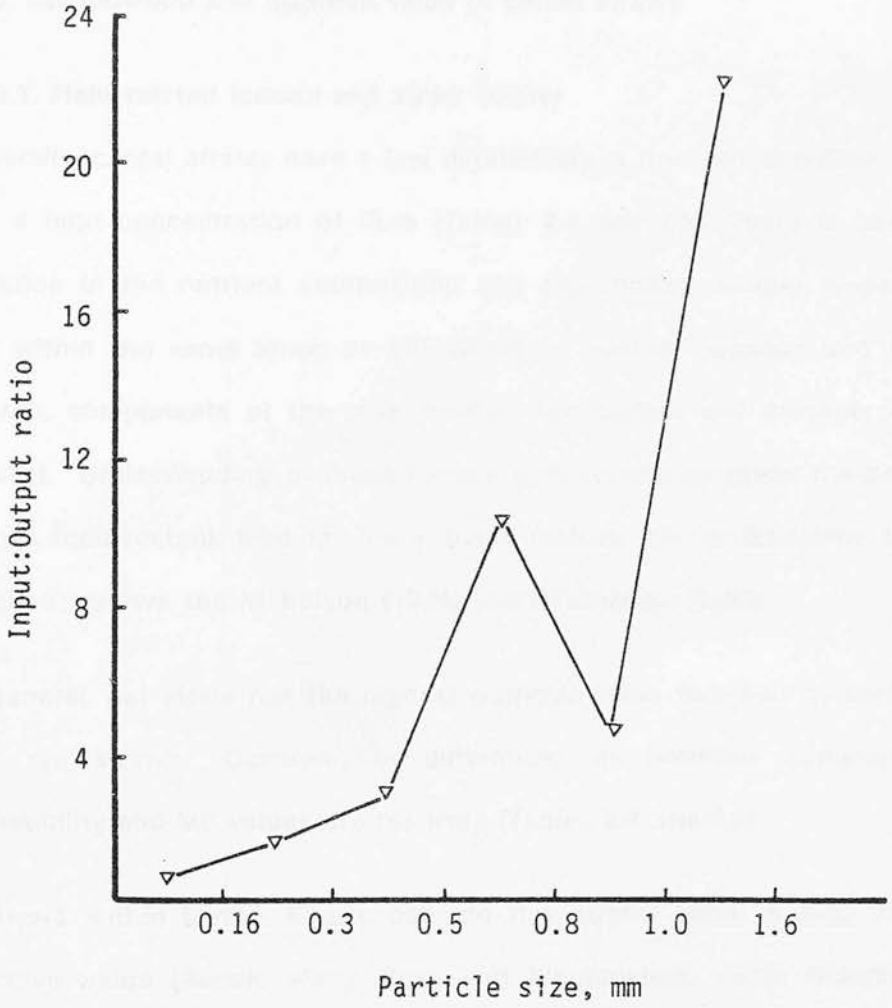

FIGURE 2.8 Relationship between the particle size in different rumen digesta pools in grazing cattle and the input: output ratio of particles for each pool (See text for details). (Adapted from Lascano, 1979). (Cited by Van Soest, 1982).

2.6. HIGHLY-DIGESTIBLE FIBROUS FEEDS AS SUPPLEMENTS TO LOW-QUALITY ROUGHAGES

2.6.1. Composition and nutritive value of cereal straws

2.6.1.1. Plant related factors and straw quality

Generally, cereal straws have a low digestibility, a low concentration of protein and a high concentration of fibre (Tables 2.4 and 2.5). There is considerable variation in the nutrient composition and digestibility, among types of straw and within the same straw as influenced by cultivar, location and season of growth, components of the crop residue, fertilisation and damage after grain harvest. Understanding of these factors is important to select the best quality straws for livestock feeding. Here, these factors will be discussed briefly; for detailed reviews see Nicholson (1984) and O'Donovan (1983).

In general, oat straw has the highest nutritive value followed by barley, wheat and rye straws. Considerable differences in chemical composition, OM digestibility and ME values are reported (Tables 2.4 and 2.5).

Cultivars within barley, wheat, oat and rice straws show a wide variation in nutritive value (Acock, Ward, Rush and Klopfenstein, 1978; Alderman, 1976; Devasia, Thomas and Nandakumaran, 1976; Erickson, Meyer and Foster, 1982; Kernan, Crowle, Spurr and Coxworth, 1979; Palmer, 1976). For example, the *in vitro* OMD of 27 cultivars of barley straw ranged from 0.3 to 0.5 in a study reported by Erickson *et al* (1982).

Between-cultivar differences in the quality of straws are often less than differences due to location and season of growth (Acock *et al*, 1978; Coxworth, Kernan, Knipfel, Thorlacius and Crowle, 1981; Erickson *et al*, 1982; Kernan *et al*, 1979). The higher feeding value of spring barley and wheat straws compared

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TABLE 2.4 Chemical composition of cereal straws.

Crop species	MJ/kg DM	g/kg DM											mg/kg DM		
	GE	CP	CF	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn
Barley															
Mean[1]	18.7	49	467	733	504	67	92	5.0	1.2	1.8	0.8	11.0	5.0	25	23
Range	16.7–20.7	21–86	298–513	606–858	457–560	51–97	28–158	1.3–7.8	0.4–3.0	0.4–3.1	0.7–1.0	7.9–14.2	5–5	20–32	16–36
n[2]	13	136	38	80	83	80	110	40	77	35	4	4	4	4	4
Wheat															
Mean	19.5	31	426	797	566	92	72	1.6	0.5	0.9	0.4	7.4	3.2	21	36
Range	17.6–20.2	15–53	379–451	756–851	496–611	79–104	31–102	1.0–5.0	0.3–0.9	0.4–1.0	0.1–0.8	3.5–16.1	3.0–4.0	20–22	12–37
n	22	51	12	19	20	19	22	54	54	54	18	19	38	2	38
Oats															
Mean	19.1	39	414	794	526	91	66	1.1	0.7	0.7	4.8	8.1	6.0	22	25
Range	17.6–21.7	9–68	339–451	767–819	461–559	53–146	39–167	0.7–1.5	0.4–1.1	0.6–0.7	1.2–8.1	5.7–10.7	5.0–7.0	18–27	22–28
n	8	45	18	4	4	7	17	4	4	4	4	4	2	2	2
Rice															
Mean	16.3	46	325	744	484	55	153	5.3	2.8	1.1	2.0	4.8	5.0	–	400
Range	15.9–16.7	22–76	279–364	742–747	459–510	45–62	106–251	0.9–6.7	1.0–5.4	–	1.4–3.0	2.5–12.0	–	–	–
n	2	16	15	2	2	3	15	13	13	1	11	12	1	–	1

1. Weighted means calculated from values for individual samples or means reported. For details, see Table 2.2A.
2. Total number of samples analyzed.

TABLE 2.5 *In vitro* and *in vivo* digestibility and metabolizable energy concentration of cereal straws.

Straw	*In vitro* (g/g)		*In vivo* (g/g)		
	OMD[1]	DOMD[2]	OMD[3]	DOMD[4]	ME(MJ/kg DM)[3/4]
Oat					
Mean	0.534	0.398	0.501	0.500	7.6
Range	0.341–0.680	0.366–0.428	0.458–0.535	0.380–0.590	5.2–10.1
n[5]	71	3	10	7	17
Barley					
Mean	0.474	0.377	0.460	0.450	6.6
Range	0.220–0.510	0.366–0.395	0.412–0.526	0.330–0.560	3.4–9.4
n	89	5	7	38	46
Wheat					
Mean	0.434	0.367	0.434	0.420	6.3
Range	0.280–0.580	0.341–0.394	0.398–0.503	0.300–0.530	3.9–10.5
n	52	6	6	34	39

1. From: Eriksson (1981), Kernan and Coxworth (1981), Mulholland *et al* (1974) and Pearce *et al* (1979).
2. From: Kernan *et al* (1979).
3. From: Table 15: Wainman *et al* (1984). Sheep.
4. From: Table 3: Givens (1984). Sheep.
5. Total number of samples analyzed; means from individual values and means reported. Weighted means for OMD only.

to the respective winter straws is well recognized (Palmer, 1976; White, Hartman and Bergman, 1981).

The proportions of the various botanical fractions in cereal straws affect their nutritive value. Leaves and flower heads (chaff) are more digestible than stems with the internode being the least digestible fraction (Åman and Nordkvist, 1983; Kernan, Coxworth, Crowle and Spurr, 1984; Thiago and Kellaway, 1982). Several mechanical processes for industrial and on-farm use have proved satisfactory to separate the different botanical fractions in straws (Davis, Greenhalgh, Boyd and Shiach, 1986; Rexen, 1978; Vind, 1984).

Yield and/or N concentration of wheat and rice straws have been increased by N fertilisation; with small effects on their digestibility (Biswas and Choudhury, 1981; Coxworth *et al*, 1981; Eriksson, 1981; Kernan *et al*, 1984). As fertilisation is costly and primarily for increasing grain production, it is doubtful whether it can be modified for the benefit of the feeding value of the crop residue.

Straw collected shortly after grain harvesting is of best quality, as losses of the more digestible fractions and leaching of nutrients due to weathering are minimized (Jackson, 1978; Pearce, Beard and Hilliard, 1979).

2.6.1.2. Animal factors and straw feeding value

When fed alone, straws are consumed by ruminants at about 15 g per kg of liveweight per day (O'Donovan, 1983). The means reported in the literature for *in vivo* OMD and ME of cereal straws fed to sheep ranged from 0.4 to 0.5 and from 6.3 to 7.6 MJ per kg DM, respectively (Table 2.5). Besides inherent plant characteristics, ruminant species and diet selectivity influence the feeding value of cereal straws.

Cattle digest low-quality roughages to a larger extent than do sheep; the

differences become smaller as the roughage quality increases (Mertens and Ely, 1982; Playne, 1978b; Prigge, Baker and Varga, 1984; Rees and Little, 1980). It is clear that this is partly due to longer mean retention times of digesta in cattle (Poppi *et al*, 1981a,b; Prigge *et al*, 1984, Rees and Little, 1980), but the rôles of intake level, rumen fermentation, and quality of the diet selected are not fully elucidated.

There is no full agreement among research workers on whether cattle or sheep have relatively higher intakes of low-quality roughages. In addition to large differences in plant species and their composition among reports, two main features complicate these comparisons: a) the units of expressing voluntary intake and; b) the physical form of the diet. Based on metabolic rate differences between sheep and cattle, Graham (1972) proposed that interspecies comparisons for feed intake should be expressed per kg $LW^{0.9}$. Playne (1978a) and Poppi *et al* (1980) found that the intake of mature grasses, expressed as g DM per kg $LW^{0.9}$, was similar for cattle and sheep. Conversely, Rees and Little (1980) found that the average intake of four tropical grasses by sheep exceeded that by cattle (32 *v.* 27 g DM per kg $LW^{0.9}$%$_d$).When expressed as g DM per kg $LW^{0.75}$, the intake of low-quality roughages (DMD below 0.65) was invariably greater for cattle than for sheep (Playne, 1978a; Prigge *et al*, 1984; Rees and Little, 1980). Whether the 0.9 or the 0.75 power is more appropriate has not been systematically investigated and regardless of the exponent used, the relative intake of digestible DM would seem to be higher for cattle than for sheep. Moreover, relative to their energy requirements, large ruminants are more efficient users of fibrous roughages as they have larger rumen capacities and faster rumination rates and can derive more maintenance energy from rumen VFA (Van Soest, 1982).

Ruminants, especially sheep and goats, select the more digestible fractions of straws and other low-quality roughages (Van Soest, 1982). The opportunity to select is expected to decline as the forage particle size decreases, since this induces faster rates of feed consumption (Kenney and Black, 1983). Wahed and Owen (1986a,b) observed that sheep and goats fed long lucerne hay and NH_3-treated straw left refusals with higher fibre and lignin and lower *in vitro* OM digestibility than the forages offered. These differences would be less evident in cattle (Van Soest,1982). In the experiments of Playne (1978a) and Prigge *et al* (1984) sheep and cattle showed no difference in the composition of the diet selected, but the forages were ground to pass 20-40 mm screens. It will be more appropriate to compare cattle and sheep in their capacity to eat and digest low-quality roughages when their adaptative feeding behaviour is fully expressed.

As yet, it is not clear whether cattle and sheep differ in their rumen fermentation. Using the dacron bag technique, Prigge *et al* (1984) could not detect differences between these species in the ability of their rumen microbes to degrade a low-quality grass hay. Poppi *et al* (1980) and Poppi *et al* (1981c) reported faster fibre digestion rates of mature grass in sheep than in cattle which were associated with a shorter mean retention time for fibre in the rumen and a resulting lower fibre digestibility. This does not support the suggestion of Playne (1978a) that an increased microbial activity in the rumen was related to a higher forage fibre digestibility in cattle than in sheep. On the other hand, Côte, Seoane and Gervais (1983) showed that the *in sacco* degradation of several mature grasses after 48 h incubation was consistently higher for cattle than for sheep.

Prediction of intake and *in vivo* digestibility of forages from simple and

inexpensive chemical analysis and/or *in vitro* digestion techniques is of major interest to animal nutritionists. Unfortunately, none of these give reliable information on the nutritive value of cereal straws for ruminants. Straws of similar proximate analyses show large variation in *in vitro* OMD and significant relationships between *in vivo* digestibility and *in vitro* digestibility or any chemical fraction in the DM have not been reported (Barber, Adamson and Altman,1984; Owen, Perry, Burt and Pearson, 1969). Differences in the quality of the sample subjected to *in vitro* digestion and the feed selected by the animal were possible reasons advanced by Olubajo, Van Soest and Oyenuga (1974). Other factors may be involved. Firstly, fine grinding of the *in vitro* sample can improve its digestibility by increasing ruptured tissues and by breaking lignin-polysaccharide bonds (Dehority and Johnson,1961; Fan *et al*, 1981; Pigden and Heaney, 1969). Secondly, in the live animal some potentially digestible straw escapes to the hindgut.

2.6.2. Nutritive value of highly-digestible fibrous feeds

There is no unique definition of highly-digestible fibrous feeds as this depends on the feeds considered. For the purpose of this discussion, they are forages and agricultural by-products containing good-quality fibre (0.45-0.85 digestibility) and at least 8 MJ of ME per kg DM, with varying levels of protein and total fibre. Emphasis is given here to temperate and tropical legume forages, sugar beet pulp and citrus pulp. Their nutritive value is discussed with reference to their potential as supplements to low-quality roughages. Both the levels and the fermentation characteristics of the nutrients in these feeds are considered.

2.6.2.1. Legume forages

The ME concentration and chemical composition of several temperate and tropical dried legume forages are given in Tables 2.6 and 2.7. Legumes have higher ME and N concentrations than cereal straws (Tables 2.4 and 2.5). Moreover, they have much higher levels of Ca, P and Mg. The potential of legume forages as supplements to cereal straws is twofold: a direct increase in the concentration of digestible nutrients in the diet and an improvement in the fermentation of the straw by the extra supply of nutrients to the rumen microbes. The higher ME in legumes than in straws results from a higher cell content including pectin, sugars and starch, and in most cases, from a higher fibre digestibility (Tables 2.6 and 2.7). Protein and NPN, pectic substances and good-quality fibre may enhance cellulolytic activity in the rumen (See Section 2.4.3.2). In fact, small additions of legume forage hays (0.1-0.15 of total dietary DM) to low quality roughage-based diets fed to cattle and sheep have increased intake and digestion of the roughage (Lane, 1982; Minson and Milford, 1967; Siebert and Kennedy, 1972). High microbial growth rates in the rumen of sheep eating legume forages have been clearly demonstrated. The highest microbial yields in sheep have been obtained with legume and grass forages (ARC, 1984). When straws were incubated *in sacco* in the rumen of sheep consuming lucerne, their potential degradabilities and digestion rates were higher and their lag times shorter than when they were incubated in the rumen of straw-fed animals (Dryden and Kempton, 1983).

The composition of the nitrogenous compounds and their degradation in the rumen for legumes and other digestible fibrous feeds have not been studied extensively. For lucerne CP, several authors have estimated the effective degradability in the rumen and the size of the rapidly soluble and insoluble but potentially degradable fractions. Mathers and Miller (1981) estimated the *in*

TABLE 2.6 Chemical composition and ME value of highly-digestible fibrous feeds.

Feed	MJ/kg DM	g/kg DM											mg/kg DM		
	ME	CP	CF	NDF	ADF	Lignin	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn
Legume forages															
Lucerne															
Mean[1]	9.8	211	232	413	304	84	100	17	2.6	3.6	2.0	16	8.4	25	37
Range	8.3-11.3	180-267	186-278	351-480	263-371	64-110	91-112	15-20	2-3	3-4.1	1.1-3	11-26	8-11	21-30	24-61
n	4	5	4	4	5	4	4	5	5	5	4	4	5	5	5
Red clover[3]	8.3	160	288	560	360	100	85	15	2.5	4.3	1.9	16	11	17	73
Ladino clover[3]	10.3	220	212	360	320	70	101	13	3.1	4.8	1.3	26	10	17	95
Leucaena leaves[3]	-	239	134	-	-	-	88	28	2.4	-	-	-	-	-	-
Leucaena leaves + stems[3]	8.1	204	195	-	-	-	89	18	1.9	4.0	0.4	31	26	46	80
Gliricidia[3]	-	228	168	-	-	-	122	24	1.7	5.8	0.9	23	-	22	60
By-products															
Dried citrus pulp															
Mean	11.3	68	114	246	180	17	47	15	1.4	1.3	0.7	8.6	8.7	12	9
Range	8.9-12.4	50-89	74-189	230-272	106-266	10-30	31-75	6.2-19	1.0-2.7	1.2-1.7	0.3-1.0	7-10.9	6-14	10-15	6-13
n	7	19	17	3	11	9	19	8	8	5	3	3	3	3	3
Sugar beet pulp															
Unmolassed[3]															
Mean	11.7	101	199	526	299	27	57	7.4	1.4	4.6	1.3	4.0	14	1.0	38
Range	11.5-12.5	97-104	196-204	480-557	278-330	20-32	56-60								
n	3	3	3	3	3	3	3								
Molassed															
Mean	12.5	128	127	309	186	23	80	5.9	0.7	1.0	4.9	17	14	22	30
Range	11.2-13.6	101-139	118-165	271-440	164-250	17-30	61-92	5.0-7.6	0.6-1.0	0.1-1.6	3.7-6.3	14.7-18.1	11-20	15-30	18-41
n	10	10	10	10	10	10	10	10	10	10	10	10	9	8	9

1. Unweighted means from individual samples and/or means reported . For details see Table 2.3A
2. Total number of means and/or individual values.
3. No range or n values given when less than three reports are included.

TABLE 2.7 Carbohydrate content and fibre digestibility of cereal straws and some highly-digestible fibrous feeds.

Feed	Sugars	Starch	Pectin	Cellulose	Hemicellulose	Reference	Fibre digestibility (g/g)[b]	Reference
Cereal straws								
Barley	65[a]	-	-	300	254	Theander and	-	
Oats	105	-	-	300	220	Åman (1978)	0.54	Mosi and
Wheat	58	-	-	270	210		0.48	Butterwoth (1985)
Legumes								
M. sativa	50-150	10-70	50-100	200-350	80-100	Van Soest (1982)	0.43	Van Soest (1982)
T. pratense	95	33	-	146	93	Moseley and Jones (1979)	0.70[c]	Moseley and Jones (1979)
T. subterraneun	122	-	76	160	61	Egan *et al* (1975)	0.72	Egan *et al* (1975)
T. tembense	-	-	-	318	78	Mosi and Butterworth (1985)	0.64	Mosi and Butterworth (1985)
By-products								
Sugar beet pulp[d]	40	0	-	255	275	Kelly (1983)	0.85	Van Soest (1982)
Citrus pulp[e]	-	-	-	190[e]	-	NRC (1984)	0.83	Van Soest (1982)

a. Water soluble fraction of the 80% ethanol extractive.
b. Total digestible nutrients arising from cellulose and hemicellulose/ total fibre.
c. Total fibre = hemicellulose + celluiose only.
d. Dried unmolassed.
e. Dried. Cellulose = ADF - Lignin.

vivo rumen degradability of CP in lucerne hay fed to sheep at about 1.4 times the maintenance energy requirements. Their value of 0.72 was similar to the values calculated from the results of Lindsay and Hogan (1972) (0.79); Nolan and Leng (1972) (0.80) and Pilgrim *et al* (1970) (0.79); but not from the combined results of Egan, Walker, Nader and Storer (1975) and Walker, Egan, Nader, Ulyatt and Storer (1975) (0.48). As the level of intake was similar in all of these experiments, other factors such as drying temperature of lucerne, differences in techniques and experimental error might have affected the results (Britton, Rock, Klopfenstein, Ward and Merrill, 1981; Goering, 1980; Mathers and Miller, 1981).

With steers fed mixed diets containing 400 g dried lucerne per kg diet, Loerch, Berger, Plegge and Fahey (1983) found that about 0.66 of the lucerne-N escaped rumen fermentation. Britton *et al* (1981) reported higher growth rates for steers fed complete diets supplemented with dried lucerne rather than with urea or soya bean meal. Krause and Klopfenstein (1978) found that both lucerne and soya bean meal in wheat straw-based diets induced higher N-retention in lambs and higher growth rates in steers than urea. These results were largely attributed to an increased flow of lucerne protein to the small intestine. However, lucerne was always included at the expense of the roughage and grain fractions in the diets. Thus, there were marked differences in the proportion of cell wall and soluble carbohydrates and in the fibre digestibility among diets which may have confounded the effects of the protein supplements.

Assuming a fractional outflow rate of undegraded protein particles from the rumen of 0.046 per h, Mathers and Miller (1981) and Miller (1980) reported almost identical values for the degradability of lucerne CP in the rumen: 0.73

and 0.71, respectively. With the results of Mathers and Miller (1981) and Miller (1980) it can be calculated (as proposed by Ørskov and McDonald, 1979) that the effective degradability of lucerne CP in the rumen is about 0.64 when the fractional outflow rate of undegraded lucerne CP from the rumen is 0.08 per h, a value that may be expected at high levels of feeding (ARC, 1984). Although the values reported by Mathers and Miller (1981) and Miller (1980) for the rumen degradability of lucerne CP were very similar, the values for the digestion rate (k) and the rapidly soluble fraction (a) in lucerne CP were very different. Mathers and Miller (1981) reported values of k=0.214 and a=0.13 whereas Miller (1980) reported values of k=0.077 and a=0.28. These differences are likely to be due to the methods of estimating these values from data on the *in sacco* degradation of lucerne CP at different incubation times. Generally, it can be concluded that lucerne is a good source of rumen degradable protein, but more information is required on its rate of digestion in the rumen and the size of its soluble and potentially degradable fractions.

Legume forages have been associated with various abnormal conditions in ruminants; *e.g.* bloat when animals consume fresh lucerne or clovers as the sole component of the diet; alopecia, cataracts and infertility with high levels of feeding of *Leucaena leucocephala* (Smolenski, Kinghorn and Balandrin, 1981). However, these should not be major problems when legume forages are fed as supplements to low-quality roughages at levels less than 0.5 of the diet.

2.6.2.2. Sugar beet pulp

The fresh residue after extraction of sugar from sugar beet is called sugar beet pulp and contains 50-100 g DM per kg. Most of this undergoes further processing before it is sold for livestock feeding as fresh pressed pulp, dried pulp and dried molassed pulp. Fresh pressed pulp results from mechanical

extraction of water, which increases the DM to 150–200 g per kg. Dried pressed pulp is obtained after artificial drying of this product to 800–900 g DM per kg whereas inclusion of beet molasses at 180–220 g per kg DM before artificial drying to similar DM produces dried molassed pulp (Kelly, 1983; Wainman, Dewey and Boyne, 1979).

The average nutrient composition of the dried sugar beet pulps is shown in Tables 2.6 and 2.7. The ME value of both dried products is almost twice the average ME of cereal straws (Table 2.5) and slightly lower than the ME of most cereal grains (12–15 MJ per kg DM, NRC, 1984; Wainman, Dewey and Brewer, 1984; Wainman *et al*, 1979). Although dried pulps are lower in N concentration than legume forages, they do contain much higher N levels than straws (18 *v.* 7 g total N per kg DM, Tables 2.4 and 2.6). The rumen degradability of CP in sugar beet pulp has been estimated to vary from 0.64 to 0.45 as the fractional outflow rate of undigested protein particles from the rumen changes from 0.02 to 0.08 per h (ARC, 1984).

The mineral composition of dried beet pulps is not markedly different from that of cereal straws (Tables 2.4 and 2.6). Dried pulps are higher in Ca and considerably higher in Cu than are cereal straws (6.6 *v.* 3.2 g Ca per kg DM and 14 *v.* 5 mg Cu per kg DM). Molassed beet pulp contains higher levels of K than do dried beet pulp and cereal straws.

Compared to cereal grains, where most of the energy is from starch and soluble carbohydrates, the high ME in dried beet pulps can be attributed to the high concentration of digestible fibre (Table 2.7). As supplements to cereal straws they may enhance the growth and activity of the fibre-digesting microbes in the rumen (See Section 2.4.3.2). As a result, the digestion of the more refractory fibre in the straws may be improved. Intake and digestion

responses to supplementation with beet pulps are discussed in Section 2.6.3.

2.6.2.3. Citrus pulp

Citrus pulp is the by-product of the fruit juice and canning industries and consist of peels, segment tows and discarded whole fruits. Seeds may be included, but they are commonly removed for aromatic oil production. Molasses extracted from the fresh pulp may be added before drying the by-products to attain 800-900 g DM per kg (Martinez-Pascual and Fernadez-Carmona, 1980a; Ross, 1981).

The composition of dried citrus pulp varies widely depending on the amount of seeds and molasses and the type of fruits included. However, most commercially available pulps are composed of unmolassed orange and grapefruit residues with a few seeds (Ammerman, 1973; Martinez-Pascual and Fernandez-Carmona, 1980a)

The average nutrient composition of dried citrus pulp is shown in Tables 2.6 and 2.7. Citrus pulps are lower in N and NDF and slightly higher in Ca than beet pulps. Citrus pulp fibre is as digestible as beet pulp fibre (Table 2.7). The ME in citrus pulp originates from the NDF fraction and the carbohydrates in the cell contents. Less energy comes from digestible fibre in citrus pulp than in beet pulp. Therefore, supplementation of straws with citrus pulp may improve rumen microbial cellulolysis to a lesser extent than supplementation with sugar beet pulps.

The protein in citrus pulp is of low *in vivo* digestibility and solubility in buffer solutions (MacGregor, Sniffen and Hoover, 1978; Martinez-Pascual and Fernandez- Carmona, 1980b). It seems that values for the rumen degradability of CP in citrus pulp have not been reported.

2.6.2.4. Fermentation characteristics

The fermentation characteristicts of a feed are likely to influence its effectiveness as a supplement to low-quality roughages. Of these, digestion rate and the size of the readily soluble and insoluble but potentially degradable fractions are important.

Examples of *in sacco* fermentation curves of cereal straws, some highly-digestible fibrous feeds and oats are given in Figure 2.9. The mean retention times of digesta (MRT) in the rumen of cattle and sheep eating high-roughage diets are also indicated in the figure.

The slow rates of digestion of untreated (k=0.032 per h) and ammonia-treated straws (k=0.034 per h) are mainly associated with a poorly-degradable fibrous fraction. Although there are inherent characteristics in the fibre which limit its degradation, this can be improved by supplying extra nutrients to the rumen microbes. The efficiency of utilisation of these nutrients by the rumen microbes and the host would depend on the synchronisation between their release and uptake. Fast release of large amounts of nutrients over a short period would be disadvantageous. About 0.6 of the oat-DM disappears in the rumen within the first 2-3 h of incubation, when the degradation rate of the straws is imperceptible. Most of the remaining insoluble but potentially degradable DM (0.15) disappears after about 16 h (Figure 2.9). This fast release of nutrients, mainly starch and soluble carbohydrates, induces serious substrate competition and impairment of cellulolytic activity in the rumen (See Section 2.4.3.1). Moreover, most of these nutrients (0.6 of total DM) are released within 0.11 or 0.05 of the MRT for sheep and cattle, respectively. On the other hand, only about 0.17 of the sugar beet pulp DM is readily soluble and disappears in the rumen within 2-3 h of incubation. The remaining

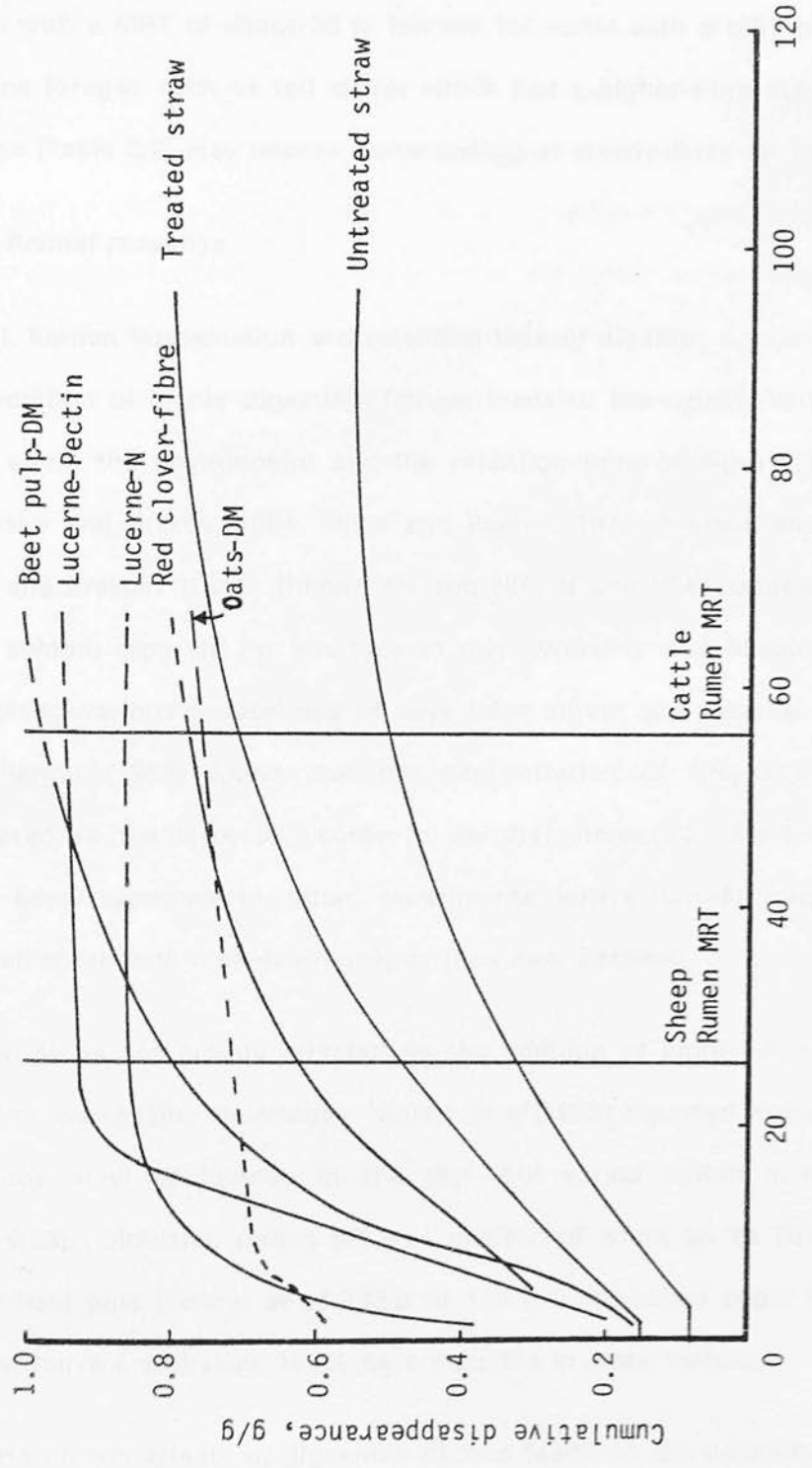

FIGURE 2.9 Disappearance of various components from feeds incubated *in saco* for different times. Beet pulp - DM (Varga and Hoover, 1983). Lucerne -N (Mathers and Miller, 1981) lucerne-pectin, red clover-fibre (Chesson and Monro, 1982). Ammonia treated and untreated barley straws (Graham and Aman, 1984). Rumen digesta mean retention times (MRT) (Van Soest, 1982; Warner, 1981). Oats - DM (Varga and Hoover, 1983).

insoluble but potentially digestible fraction (0.87) is degraded at a steady rate (k=0.055 per h) for 60-65 h. Lucerne-N and pectic substances are almost completely degraded after 20-30 h of incubation. This may be adequate for sheep with a MRT of about 26 h, but not for cattle with a MRT of about 56 h. Legume forages such as red clover which has a higher fibre digestibility than lucerne (Table 2.7) may release some energy at steady rates for up to 60 h.

2.6.3. Animal response

2.6.3.1. Rumen fermentation and retention time of digesta

The addition of highly-digestible fibrous feeds to low-quality roughage-based diets alters the environment and the retention time of digesta in the rumen (Chesson and Ørskov, 1984; Milne and Bagley, 1976; Preston and Leng, 1984; Silva and Ørskov, 1985). Rumen pH and NH_3-N and VFA concentrations have been seldom reported for this type of diet. Working with sheep offered diets containing various proportions of soya bean stover and lucerne, Soofy, Fahey and Berger (1983) found that the concentration of NH_3-N in the rumen increased as the level of lucerne in the diet increased. Similar trends may have been expected in other experiments where low-N roughages were supplemented with high-N roughages (See next Section).

Rumen pH is not greatly affected by the addition of highly-digestible fibrous feeds to low-quality roughages. Soofy *et al* (1983) reported that pH decreased with the level of lucerne in the diet, but varied within a narrow range (6.54-6.96). Similarly, rumen pH was unaffected when up to 700 g molassed sugar beet pulp (Fahmy *et al*, 1984) or 150 g unmolassed sugar beet pulp per kg diet (Silva and Ørskov, 1985) were included in straw rations.

Reports on the effects of digestible fibrous feeds on the cellulolytic activity in

the rumen are conflicting. Silva and Ørskov (1985) found that the *in sacco* degradation of untreated barley straw was significantly lower in the rumen of sheep fed straw alone than in sheep fed straw supplemented with 150 g unmolassed sugar beet pulp per kg diet (0.463 *v.* 0.503 at 48 h of incubation). Soofy *et al* (1983) reported that the *in sacco* degradation of NaOH-treated cotton thread increased markedly with the level of lucerne in soya bean-stover diets fed to sheep. On the other hand, Fahmy *et al* (1984) found that the *in sacco* degradation of NH_3-treated straw in the rumen of sheep offered treated straw supplemented with molassed sugar beet pulp was unaffected by levels of beet pulp up to 450 g per kg diet. At higher levels the straw degradation after 16 h was significantly reduced. The following may partly explain these results. Firstly, the cellulolytic activity in the rumen of sheep fed NH_3-treated straw may have already reached a maximum, which could not have been enhanced further by the addition of good quality fibre from the beet pulp. Indeed, Silva and Ørskov (1984) found that the *in sacco* degradations of untreated straw and hay at 48 h of incubation were significantly higher for sheep fed NH_3-treated straw than for sheep fed untreated straw. Secondly, at high levels of supplementation, the sugar from the molasses in the beet pulp may have induced substrate competition affecting cellulolysis (Mould *et al*, 1983).

An improved cellulolytic activity in the rumen does not always result in increased intake and *in vivo* digestibility of roughages. This may be associated with many interacting factors including the MRT of digesta in the gastrointestinal tract. Milne and Bagley (1976) found that as the proportion of good-quality grass in the diet increased, the MRT of heather (*Calluna vulgaris*) in the gut of sheep decreased linearly. There were no associative effects of grass and heather on *in vivo* DMD of the total diet. Therefore, the potentially

beneficial effect of grass supplementation on the digestion of fibre in heather may have been overriden by its shorter MRT. Paterson, Klopfenstein and Britton (1982) reported positive associative effects between ground NaOH-treated maize cobs and chopped lucerne hay on total DMD. This was partly attributed to an effect of lucerne hay on extending the MRT of the maize cobs.

2.6.3.2. Intake and digestion

Literature data on intake and *in vivo* digestibility in sheep and cattle offered diets with varying proportions of low-quality roughages and digestible fibrous feeds are summarized in Tables 2.8 and 2.9. There is considerable variation among reports concerning the nutritive value of the feeds, the breed and size of the animals, the physical form of the roughages and other experimental factors. However, some general trends will be discussed.

Low levels of high-quality roughage supplementation (100-200 g per kg total dietary DM) have had varied effects on intake of the basal roughage, *i.e.* an increase, no change or a decrease (Table 2.8). This is likely to have resulted from a combination of many factors, but the protein/energy ratio appears important. The ME values in Table 2.8 were estimated as indicated and may not be very precise in some cases, but they are included because of their usefulness in allowing a discussion of the literature on a general rather than on an individual-experiment basis. The RDP/ME ratios (g per MJ) for the diets (Table 2.8) were calculated assuming the following:

- Low-quality roughages CP degradability: 0.5 (Dryden and Kempton, 1983). High-quality roughage CP degradability: 0.7 (See Section 2.6.2.1)

TABLE 2.8 Intake and digestion in cattle and sheep fed low quality roughages with different proportions of highly digestible fibrous feeds.

Animal species (LW)	Low quality roughage (LQR)		High quality fibrous feed			Total diet DM		DM intake, $g/kg^{0.75}$/day		Diet digest, g/g		N retained	Reference, comments
	Species, form and feeding level	CP g/kg DM	Species, form and feeding level	CP g/kg DM	DM in total ration, g/g	RDP/ ME	ME, MJ/kg	LQR	Total	DM	OM	g/day	
Sheep (20 kg)	*Hyparrhenia rufa* Flail harvested standing hay *Ad libitum*	13	*Macroptilium stropurpureum* Long, fresh at fixed levels Expt 1	143	0	2.8	5.6[1]	53	53^{a}	0.43			Lane (1982). 1 Estimated as ME = (0.98 x DMD - 4.8) X 0.15 (MAFF, 1984) Intakes calculated with the mean sheep weight to the 0.75 power Statistical analysis done with intake as g/kg LW/day
					0.16	4.8	6.0	57	68^{b}	0.46			
					0.32	7.1	6.2	44	65^{b}	0.47			
					0.54	10.6	6.2	27	59^{b}	0.47			
					0.56	10.1	6.5	32	72^{b}	0.49			
					0.73	12.4	6.6	19	70^{b}	0.50			
	As above	13	*Stylosanthes guyanensis* Long, fresh at fixed levels Expt 2	85	0	3.0	5.3	53	53^{a}	0.41			
					0.19	3.8	6.3	53	65^{b}	0.48			
					0.40	5.2	6.9	38	63^{b}	0.52			
					0.53	5.9	7.2	30	63^{b}	0.54			
					0.62	7.0	6.6	27	72^{b}	0.50			
					0.86	9.2	6.6	9	59^{b}	0.50			
Sheep (25.4 kg)	*Zea mays* Maize stover *Ad libitum*	51	*Trifolium tembense* Hay at fixed levels Expt 1	201	0	4.7	7.4	44^{a}	44^{a}	0.54^{a}	0.56^{a}	0.04^{a}	Mosi and Butterworth (1985). ME=DE x 0.81 (MAFF, 1984). Intakes calculated as above
					0.24	7.3	8.6	32^{bc}	44^{a}	0.61^{b}	0.63^{b}	2.38^{b}	
					0.35	8.3	8.9	34^{b}	53^{b}	0.63^{b}	0.66^{b}	4.42^{c}	
					0.51	10.0	9.2	27^{b}	53^{b}	0.65^{b}	0.66^{b}	3.54^{c}	
	Avena sativa Oat straw *Ad libitum*	62	As above Expt 2	201	0	5.0	7.6	60^{a}	60^{a}	0.52^{a}	0.55^{a}	2.79^{a}	
					0.14	6.5	8.2	51^{b}	60^{a}	0.57^{b}	0.59^{b}	3.10^{b}	
					0.24	7.3	8.7	51^{b}	67^{b}	0.60^{c}	0.63^{c}	5.45^{b}	
					0.36	8.8	8.7	43^{c}	67^{b}	0.60^{c}	0.62^{c}	5.54^{b}	
	Eragrostis tef Tef straw *Ad libitum*	36	As above Exp 3	201	0	2.9	6.3	52^{a}	52^{a}	0.45^{a}	0.49^{a}	-1.50^{a}	
					0.19	6.7	7.1	51^{a}	63^{b}	0.48^{a}	0.52^{a}	-0.03^{a}	
					0.36	8.7	7.8	44^{b}	68^{c}	0.52^{ab}	0.55^{ab}	0.46^{ab}	
					0.50	10.2	8.3	36^{c}	72^{d}	0.56^{b}	0.58^{b}	1.75^{b}	
	Triticum spp Wheat straw *Ad libitum*	23	As above Expt 4	201	0	3.1	6.4	47^{a}	47^{a}	0.40^{a}	0.47	-0.51^{a}	
					0.19	6.2	7.1	44^{a}	54^{ab}	0.42^{a}	0.48	0.41^{b}	
					0.34	8.3	7.6	37^{b}	57^{b}	0.46^{ab}	0.51	2.42^{c}	
					0.44	9.9	7.7	35^{b}	62^{b}	0.48^{b}	0.53	3.56^{d}	

Contd. TABLE 2.8

Animal species (LW)	Low quality roughage (LQR)		High quality fibrous feed		Total diet DM			DM intake, $g/kg^{0.75}$/day		Diet digest, g/g		N retained	Reference, comments
	Species, form and feeding level	CP g/kg DM	Species, form and feeding level	CP g/kg DM	DM in total ration, g/g	RDP/ ME*	ME, MJ/kg	LQR	Total	DM	OM	g/day	
Sheep (41–56 kg) Expt 1	*Digitaria decumbens* Pangola grass Chopped hay 1.3–2.5 cm *Ad libitum*	44	*Medicago sativa* Lucerne Chopped hay 1.3–2.5 cm At fixed levels Expt 1	225	0	4.3	6.3	49	49	0.48			Minson and Milford (1967). ME=(10.98xDMD−4.8)x0.15 MAFF (1984). Statistical analysis not fully reported
					0.10	6.1	6.7	47	53	0.50			
					0.20	7.8	7.0	44	55	0.53			
					0.36	10.0	7.5	40	61	0.56			
					1.00	–	9.4	0	90	0.69			
(41–56 kg) Expt 2	As above	36	As above Expt 2	225	0	3.6	7.2	33	33	0.54			
					0.11	5.0	7.7	43	48	0.58			
					0.19	6.2	7.9	46	57	0.58			
					0.37	9.1	8.1	37	59	0.60			
					1.00	–	9.3	0	83	0.68			
(41–56 kg) Expt 3	As above	36	*Trifolium repens* Ladino clover As above Expt 3	250	0	3.5	7.3	35	35	0.54			
					0.11	5.1	7.9	46	51	0.59			
					0.20	6.4	8.4	43	54	0.62			
					0.37	9.3	8.6	37	59	0.63			
					1.00	–	10.4	0	70	0.76			
Sheep (37.1 kg)	*Heteropon contortus* Spear grass Hay, *Ad libitum* Form N.R.	42	*Medicago sativa* Lucerne Hay, at fixed levels Form N.R.	194	0	6.6	4.9	27^{a}	27^{a}	0.34^{a}		−1.9	Siebert and Kennedy (1972) ME=DEx0.81 ;MAFF (1984) [2]Sheep 15 g/d; Cattle=60 g/day Statistical analysis on intake as g/head per day
					0 + urea[2]	–	4.7	27^{a}	27^{a}	0.33		0.1	
					0.14	7.6	5.9	35^{ac}	41^{b}	0.42^{b}		0.2	
					0.24	9.5	5.8	37^{c}	49^{c}	0.43^{bc}		0.8	
					0.44	12.4	6.4	30^{ac}	54^{c}	0.48^{c}		3.9	
Cattle (142 kg)	As above	42	As above	194	0	3.8	6.1	52	52	0.44		1.9	
					0 + urea[2]	–	6.1	51	51	0.45		4.0	
					0.14	6.6	5.9	62	71	0.44		3.3	
					0.24	8.5	5.9	60	80	0.43		7.2	
					0.45	12.3	6.0	48	87	0.45		12.8	
Sheep (43 kg)	*Calluna vulgaris* Heather Flail-harvested Fresh after freeze-storage. *Ad libitum*	78	*Lolium perenne* *Trifolium repens* (94%/5%) (Perennial rygrass/white clover) Fresh after freeze storage. At fixed levels	133	0	7.9	6.4	24	24	0.44^{3}	0.44^{3}	−2.6	Milne and Bagley (1976) ME=(10.98xDMD−4.8)x0.15 MAFF (1984). [3]Significant linear effect
					0.32	8.4	7.5	29	42	0.51	0.52	−0.4	
					0.55	9.0	8.3	21	45	0.58	0.59	0.4	
					0.74	9.4	9.0	14	51	0.63	0.64	2.1	
					0.81	9.4	9.3	11	56	0.66	0.67	2.6	

Contd. TABLE 2.8

Animal species (LW)	Low quality roughage (LQR)		High quality fibrous feed			Total diet DM		DM intake, $g/kg^{0.75}$/day		Diet digest, g/g		N retained	Reference, comments
	Species, form and feeding level	CP g/kg DM	Species, form and feeding level	CP g/kg DM	DM in total ration, g/g	RDP/ ME*	ME, MJ/kg	LQR	Total	DM	OM	g/day	
Sheep (35 kg)	*Hordeum vulgare* Barley straw Shredded 2.5 cm+ Urea[4]. *Ad libitum*	42	Species N.R. Dried grass Shredded 2.5 cm At fixed levels	147	0	-	6.7	43[a]	43[a]	0.51[a]			Mbatya *et al* (1983a) [4]2.3 g urea and 11 g molasses/100 g straw ME=(0.98xDMD-4.8)x0.15; MAFF(1984)
					0.27	-	6.9	36[b]	49[b]	0.52[a]			
					0.53	-	7.8	27[c]	57[c]	0.58[a]			
As above	As above+molasses+ urea[3]		As above		0	-	7.0	47[a]	44[a]	0.49[a]			
					0.26	-	7.6	38[b]	51[b]	0.54[a]			
					0.54	-	8.5	29[c]	62[c]	0.55[a]			
Sheep (37 kg)	*Hordeum vulgare* Barley straw Shredded 2.5 cm + Urea-molasses[5]	88	Species N.R. Dried grass Shredded 2.5 cm At fixed levels	151	0.25		6.8	35	47	0.51			Mbatya *et al* (1983b) [5]As Mbatya *et al* (1983a) ME=(0.98xDMD-4.8)x0.15 MAFF (1984)
					0.57		8.4	24	56	0.62			
					0.81		9.1	12	66	0.67			
Cattle (222 kg)	Mixture of species Fresh, chopped *Ad libitum*	88	*Leucaena leucocephala* Dried, leaves + young stems, chopped 3-4 cm lenghts	223	0	6.2	7.3	78	78[a]	0.42		13.6[a]	Wahyuni *et al* (1982) ME=DEx0.81; MAFF (1984)
					0.20	8.8	7.6	74	93[b]	0.44		31.5[b]	
					0.40	11.9	7.5	58	96[b]	0.46		38.8[b]	
					0.60	16.0	7.0	38	94[b]	0.44		42.8[cb]	
					1.00	-	8.1	0	75[c]	0.51		53.9[c]	
Sheep (BW, N.R.)	Species N.R. Ammonia-treated straw. Form N.R. *Ad libitum*	81	Molassed dried beet pulp + 10 g urea/kg Form N.R.	106	0			36	36	0.63[6]			Fahmy *et al* (1984) [6]*In sacco* dissappearance of NH_3-straw at 48 h incubation
					0.24			41	54	0.61			
					0.37			44	70	0.63			
					0.48			39	75	0.59			
					0.59			29	71	0.57			
					0.70			23	76	0.47			
Sheep (BW, N.R.)	*Hordeum vulgare* Barley straw *Ad libitum*		Unmolassed dried beet pulp + urea	N.R.	0			414[7]		0.46[8]			Silva and Ørskov (1985) [7]g DM per sheep per day [8]*In sacco* dissappearance of untreated straw at 48 h incubation
					0.15			505		0.50			

*See text for details (Section 2.6.3.2).
N.R.: not reported
a,b,c: means with different superscripts are significantly different as reported in each publication.

TABLE 2.9 Literature data on intake and digestion in sheep fed mixed diets with low and high quality roughages.

Animal species and liveweight	Low quality roughage		High quality roughage			Total diet Form and feeding level	Total diet dry matter			Reference
	Species and form	CP g/kg DM	Species and form	CP g/kg DM	DM in diet, g/g		CP g/kg	Intake g/kg $LW^{0.75}$ per day	Digest. g/g	
Sheep 31.8 kg	*Zea mays* NH_3-treated maize cobs Form N.R.	N.R.	*Medicago sativa* Lucerne hay Form N.R.	173	0 0.15 0.30 1.00	Form N.R. *Ad libitum*		33[1] 73 78 105	0.49[1,2] 0.62 0.69 0.62	Brandt and Klopfenstein (1984) [1]Significant linear effects [2]DMD at 0.9 of *ad libitum* intake N.R.: not reported
	As above		As above	149	0 0.15 0.30 1.00	As above		33[1] 59 76 78	0.49[1,2] 0.56 0.63 0.47	Intake of maize cobs alone measured only once
	As above		Brome grass Form N.R.	108	0 0.15 0.30 1.00	As above		33[1] 56 67 100	0.49[1,2] 0.52 0.62 0.62	
	As above		As above	79	0 0.15 0.30 1.00	As above		33[1] 54 65 74	0.49[1,2] 0.51 0.58 0.50	
Sheep 33 kg	*Festuca arundinacea* Tall fescue hay Ground 0.95-cm screen	69	*Medicago sativa* Lucerne hay Ground 0.95-cm screen	190	0 0.25 0.50 0.75 1.00	Mixed diet *Ad libitum*	69 99 129 160 190	39[1] 50 52 56 59	0.53[3] 0.56 0.57 0.56 0.54	Hunt *et al* (1985) [1]Significant linear effect [3]Significant quadratic effect
	As above		As above		0 0.25 0.50 0.75 1.00	Mixed diet restricted*	As above		0.55[1] 0.56 0.57 0.57 0.57	*33 g per kg $LW^{0.75}$ per day

Contd. Table 2.9

Animal species and liveweight	Low quality roughage		High quality roughage			Total diet Form and feeding level	Total diet dry matter			Reference
	Species and form	CP g/kg DM	Species and form	CP g/kg DM	DM in diet, g/g		CP g/kg	Intake g/kg $LW^{0.75}$ per day	Digest. g/g	
Sheep 37 kg Expt 2	*Hordeum vulgare* Barley straw + urea-molasses[4] Shredded 2.5-cm screen	88	Species N.R. Dried grass shredded 2.5-cm screen	151	0	Mixed diet *Ad libitum*	88	40[1]	0.47[1]	Mbatya *et al* (1983b) [1]Significant linear effects [4]2.3 g urea + 11 g molasses per 100 g straw
					0.21		126	50	0.49	
					0.50		143	69	0.59	
					0.75		139	84	0.67	
					1.00		151	90	0.73	
Expt 1	As above		As above		0.23	As above	127	54[1]	0.54[1]	
					0.52		144	63	0.58	
					0.75		139	78	0.66	
Sheep 40 kg	*Zea mays* Maize cobs, ground 0.65 cm screen	N.R.	*Medicago sativa* Lucerne hay chopped 3-cm lenght + urea/blood meal[5]	N.R.	0	Mixed diet *Ad libitum*		38[2]	0.60[6]	Paterson *et al* (1982) [2]Significant linear effect [3]Significant quadratic effect [5]1:1 urea-N:blood meal-N to attain 110 g CP/kg DM [6]Significant linear, quadratic and cubic effects, but not clear tendency [*]42 g per kg $LW^{0.75}$ per day
					0.25			41	0.55	
					0.50			50	0.58	
					0.75			55	0.55	
					1.00			71	0.50	
	As above		As above		As above	Mixed diet restricted[*]			0.60[2]	
									0.63	
									0.56	
									0.55	
									0.49	
	As above, but NaOH-treated		As above		As above	Mixed diet *Ad libitum*		72[3]	0.59[3]	
								91	0.62	
								84	0.64	
								75	0.58	
								73	0.50	
	As above		As above		As above	Mixed diet restricted[*]			0.59[3]	
									0.68	
									0.64	
									0.39	
									0.49	

Contd. Table 2.9

Animal species and liveweight	Low quality roughage		High quality roughage			Total diet Form and feeding level	Total diet dry matter			Reference
	Species and form	CP g/kg DM	Species and form	CP g/kg DM	DM in diet, g/g		CP g/kg	Intake g/kg/LW$^{0.75}$	Digest. g/g	
Sheep 40 kg	Maize stalks Chopped to 5-cm lenghts	N.R.	Lucerne hay Chopped 3 cm Lenghts + urea blood meal[5]	N.R.	0 0.25 0.50 0.75 1.00	Mixed diet *Ad libitum*		34[7] 40 50 58 48	0.62[7] 0.56 0.50 0.50 0.45	Paterson *et al* (1982) [5]See previous page. [7]Non-significant effects
	As above		As above		As above	Mixed diet restricted*			0.61[7] 0.56 0.50 0.50 0.45	*26 g per kg LW$^{0.75}$ per day
	As above, but NaOH-treated		As above		As above	Mixed diet *Ad libitum*		45[7] 51 66 64 50	0.54[7] 0.61 0.55 0.52 0.50	
	As above		As above		As above	Mixed diet restricted*			0.69[7] 0.61 0.52 0.52 0.45	
Sheep 60 kg	*Glycine max.* soya bean stover, ground 0.95-cm screen	57	*Medicago sativa* lucerne hay, ground 0.95 cm screen	195	0 0.33 0.67 1.00	Mixed diet 0.9 of *ad libitum*	57 102 149 195	54 70 101 106	0.40 0.45 0.52 0.58	Soofy *et al* (1982) No associative effects on intake and digestibility

- No great changes in the outflow rate of protein particles from the rumen between feeding levels of 0.5–1.0 times the maintenance energy requirements (0.01–0.02 per h) (Eliman and Ørskov, 1984b).

- 0.75 (0.4–1.1) g urea-N per day recycled into the rumen when low-quality roughages form a high proportion of the diet (MacRae, Milne, Wilson and Spence, 1979; Norton, Moran and Nolan, 1979). No increased recycling was considered with high levels of high-quality roughage supplementation.

In the experiments of Lane (1982), Milne and Bagley (1976), Mosi and Butterworth (1985), Minson and Milford (1967) and Siebert and Kennedy (1972) (Table 2.8), small additions of high-quality roughages maintained or increased the intake of the basal roughages; these had RDP/ME ratios below 7.9, which did not reach the minimum microbial requirements of 8.4 g RDP per MJ ME (ARC, 1984). However, in some cases low levels of supplementation decreased the intake of the basal roughage despite an apparent RDP deficiency (Mosi and Butterworth, 1985, experiments 1 and 2; Wahyuni, Yulianti, Komara, Yates, Obst and Lowry, 1982). Here the beneficial effects of adding nutrients from the legume forages may have been counteracted by rumen fill limitations and microbial substrate preferences.

Levels of high-quality roughages above 200 g per kg total dietary DM almost invariably depressed intakes of the basal roughage, regardless of the RDP/ME ratio in the diet (Table 2.8). Data from experiments with sheep showing such a decrease were divided into two groups according to the level of total DM intake: high ($>$ 45g per kg $LW^{0.75}$) and low ($<$ 45g per kg $LW^{0.75}$). Linear regressions of total digestible DMI on high-quality roughage DMI and of low-quality roughage DMI on high-quality roughage DMI were calculated for each level of intake (Figures 2.10 and 2.11). Despite the between-experiment variation, the data showed very clear trends. For high levels of intake (Figure 2.10), the replacement rate was 0.77 (reduction in low-quality DMI for each

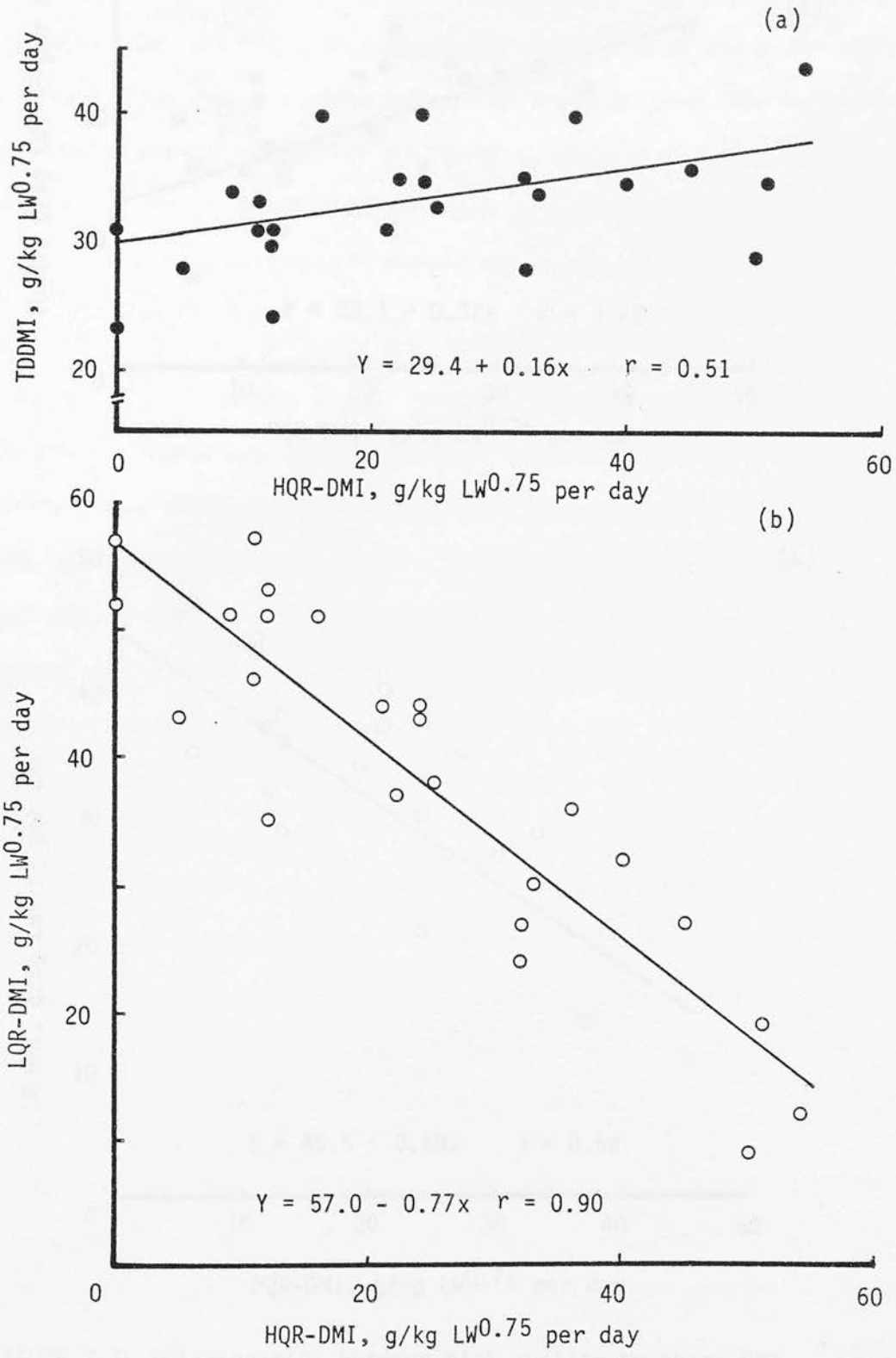

FIGURE 2.10 Relationships between high quality roughage DMI (HQR-DMI) and (a) total digestib le DMI (TDDMI); (b) low quality roughage DMI (LQR-DMI) for high levels of intake (See text and Table 2.8 for details).

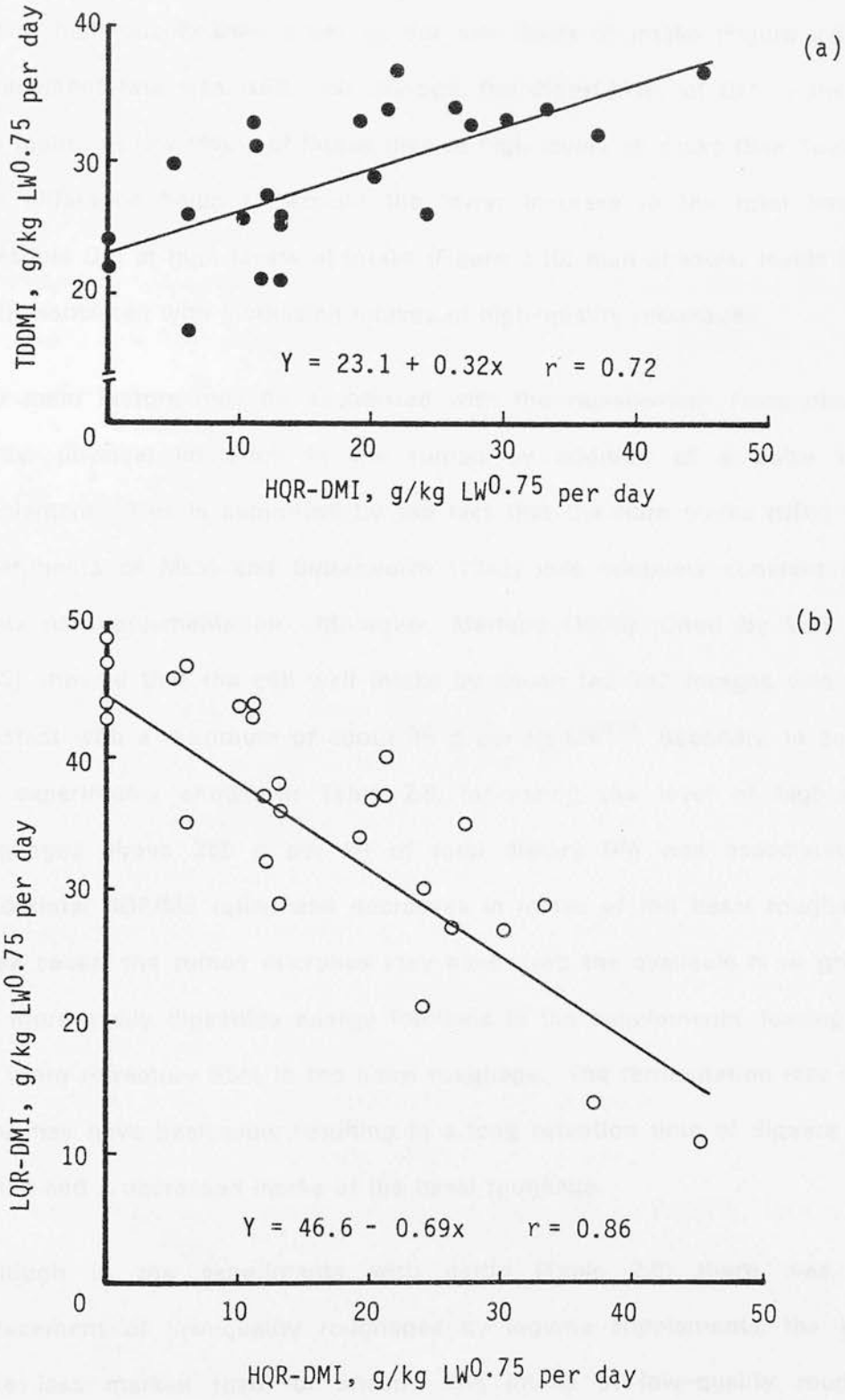

FIGURE 2.11 Relationships between high quality roughage DMI (HQR-DMI) and (a) total digestible DMI (TDDMI); (b) low quality roughage DMI (LQR-DMI) for low levels of intake (See text and Table 2.8 for details).

unit of high-quality DMI, g per g). For low levels of intake (Figure 2.11) the replacement rate was 0.69. On average, the digestibility of DM in the diets was higher at low levels of intake than at high levels of intake (See Table 2.8). This difference helps to explain the lower increase in the total intake of digestible DM at high levels of intake (Figure 2.10) than at lower levels (Figure 2.11) associated with increasing intakes of high-quality roughages.

Two main factors may be associated with the replacement rates observed. Firstly, physical limitation in the rumen by addition of a bulky fibrous supplement. This is supported by the fact that the fibre intake (NDF) in the experiments of Mosi and Butterworth (1985) was relatively constant across levels of supplementation. Moreover, Mertens (1973) (Cited by Van Soest, 1982) showed that the cell wall intake by sheep fed 187 forages was nearly constant with a maximum of about 35 g per kg $LW^{0.75}$. Secondly, in some of the experiments shown in Table 2.8, increasing the level of high-quality roughages above 200 g per kg of total dietary DM was associated with suboptimal RDP/ME ratios and decreases in intake of the basal roughage. In these cases, the rumen microbes may have used the available N to grow on the more easily digestible energy fractions in the supplements, leaving aside the more refractory fibre in the basal roughage. The fermentation rate of this fibre may have been slow resulting in a long retention time of digesta in the rumen and a decreased intake of the basal roughage.

Although in the experiments with cattle (Table 2.8) there was some replacement of low-quality roughages by legume supplements, the effects were less marked than for sheep. The intake of low-quality roughages supplemented with about 400 g of legume DM per kg of total dietary DM was 0.7-0.9 of the the intake of the unsupplemented roughages. A similar level of

supplementation with high-quality roughages in sheep reduced the intake of low-quality roughages to about 0.4 of the intake of the unsupplemented low-quality roughages (Figures 2.10 and 2.11).

There are only a few reports on the use of sugar beet pulp as a supplement to low-quality roughages (Table 2.8). Supplementation of NH_3-treated straw with molassed sugar beet pulp up to 450 g per kg of the total dietary DM had little or no effect on straw intake by sheep, but higher levels of supplementation reduced straw intake (Fahmy *et al*, 1984). Working with sheep offered urea-supplemented barley straw, Silva and Ørskov (1985) found a significant increase in straw intake by feeding 150 g of unmolassed sugar beet pulp per kg of total dietary DM (414 *v.* 505 g straw DM per sheep per day).

Responses in intake and digestibility to supplementation of low-quality roughages with high-quality forages in mixed diets for ruminants have been variable (Table 2.9). Positive associative effects between NaOH-treated maize cobs and chopped lucerne on intake and *in vivo* DMD were reported by Paterson *et al* (1982). The total digestible DMI was significantly higher for diets with 250-500 g of lucerne per kg of diet than for the NaOH-treated maize cobs or lucerne alone. These effects were attribut ed to several factors that may have enhanced fibre digestion in the rumen, *e.g.* an increase in the mean retention time of the cobs; dilution of the concentration of Na in the diet which at increased levels can depress cellulose digestibility; and addition of minerals by the lucerne. Improvement of rumen fermentation seemed to be the main reason for the positive associative effects between tall fescue and lucerne hays on DMD when both feeds were finely ground (Hunt, Paterson and Williams, 1985). However, these results were obtained with *ad libitum* intakes and may have been confounded by the negative effects of an increasing intake

on digestibility of forages.

In most experiments summarized in Table 2.9, positive associative effects between the high and low-quality roughages on intake and or DMD of the mixed diet were not detected. In all cases, total DMI and total digestible DMI increased linearly with the level of high-quality roughage supplementation. The corresponding data were divided according to the level of total DMI: high (46-93 g per kg $LW^{0.75}$ per day) and low (39-57 g per $kg^{0.75}$ per day). Linear regressions of total DMI on high-quality roughage DMI and total digestible DMI on high-quality roughage DMI were calculated for each level of intake (Figure 2.12). At low levels of intake, total DMI and total digestible DMI increased by about 0.37 and 0.15 g, respectively, for each g of high-quality roughage DM consumed. At high levels of intake, the corresponding increases in total DMI and total digestible DMI were 0.48 g and 0.33 g. These results indicate that at both levels of intake these was a high replacement rate (0.52-0.63).

2.6.3.3. Animal performance

As shown in Tables 2.8 and 2.9 the intake of CP and digestible DM increase with increasing the level of supplementation with high-quality roughages. Therefore, animal performance would be expected to increase.

Generally, sheep and cattle increase their N retention as the level of high-quality roughage in the diet increases. However, results for N retention should be interpreted with caution. They are usually overestimated due to inevitable losses of N from urine and faeces during handling and storage and losses of N in hair, wool and scurf which are not taken into account in conventional balance trials. Supplementation levels required to achieve positive N retentions in sheep varied from 140 to 550 g per kg of total dietary DM (Table 2.8). Devendra (1983) found that a diet of rice straw supplemented

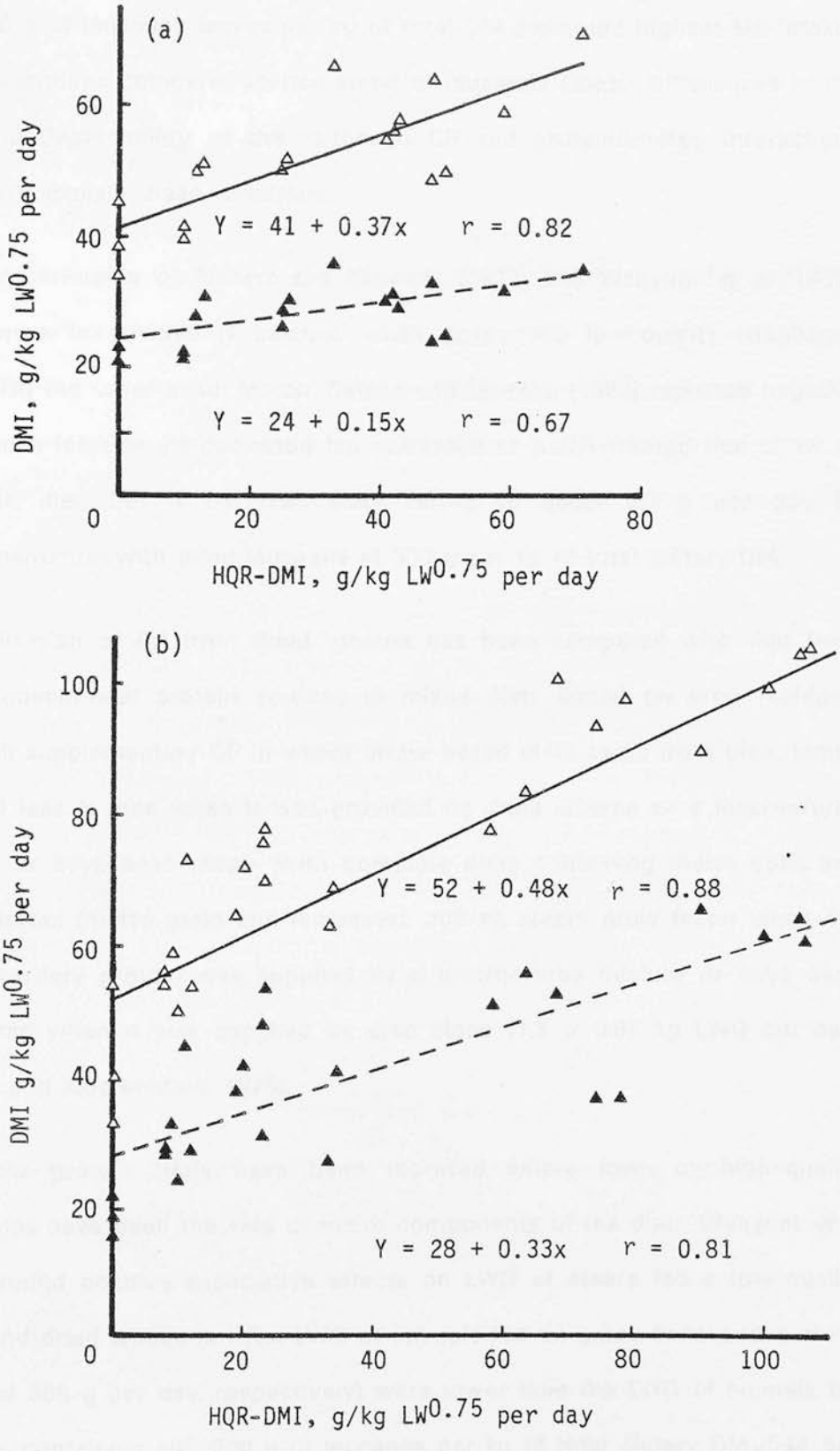

FIGURE 2.12 Relationships between high quality roughage DMI (HQR-DMI) and total DMI (——) or total digestible DMI (---) for (a) low levels of intake and (b) high levels of intake of mixed diets (See text and Table 2.9 for details).

with 400 g of leucaena leaves per kg of total DM promoted highest ME intakes and N retentions compared to rice straw or leucaena alone. Differences in the level and degradability of the roughage CP and protein/energy interactions may partly explain these variations.

In the experiments of Siebert and Kennedy (1972) and Wahyuni *et al* (1982), cattle were in positive N balance when consuming low-quality roughages alone. On the other hand, Moran, Satoto and Dawson (1983) reported negative N balances for *Bos indicus* cattle fed untreated or NaOH-treated rice straw as the sole diet, but N balances were raised to about 20 g per day by supplementation with dried leucaena at 300 g per kg of total dietary DM.

The utilisation of CP from dried lucerne has been compared with that from more conventional protein sources in mixed diets based on crop residues. When all supplementary CP in wheat straw-based diets came from urea, lambs retained less N than when it was provided by dried lucerne or a lucerne/urea mixture or soya bean meal. With complete diets containing maize cobs and concentrates (maize grain and molasses), 200-kg steers grew faster when the supplementary protein was supplied by a lucerne/urea mixture or soya bean meal than when it was supplied by urea alone (1.3 *v.* 0.97 kg LWG per day) (Krause and Klopfenstein, 1978).

Very few growth trials have been reported where low- or high-quality roughages have been the sole or major components of the diet. Wahyuni *et al* (1982) found positive associative effects on LWG of steers fed a low-quality grass and dried leucaena. The LWG of animals fed on grass or leucaena alone (-15 and 306 g per day, respectively) were lower than the LWG of animals fed on diets containing 400-600 g of leucaena per kg of total dietary DM (544-587 g per day). Similar effects were found by Paterson *et al* (1982) for steers fed

diets based on maize cobs. Animals consuming a diet with a ratio of maize cobs: lucerne of 1:1 gained 510 g per day whereas those fed on cobs plus urea and blood meal or lucerne alone gained 390 and 360 g per day, respectively.

In sheep, LWG was increased by supplementing a low-quality grass hay with 500-750 g of lucerne hay per kg of total dietary DM (Bowman and Asplund, 1984). Survival of ewes (%), birthweight of lambs (kg) and weight of lambs at 15 weeks of age (kg per ewe served) increased from 50%, 0.9 kg and 1.3 kg to 100%, 2.0 kg and 11 kg, respectively, as the legume forage (*Gliricidia maculata*) fed with a low-quality grass (*Brachiaria muliformis*) increased from 0 to 750 g per kg of diet on a fresh basis (Chadhokar and Kantharaju, 1980). A stimulatory effect of small amounts of fresh green forage on rumen fermentation and performance of ruminants fed fibrous crop residues has also been reported (O'Donovan, 1983; Verma and Jackson, 1984; Preston and Leng, 1984). Singh (1980) (Cited by Preston and Leng, 1984) showed that heifers consuming a basal diet of wheat straw and sugar cane tops grew faster when offered 0.9 kg DM per head per day of a good-quality water plant (*Azolla spp*) than 1.5 kg DM per head per day of a concentrate (maize, ricebran, groundnut cake) (330 *v.* 140 g LW per day). Small additions of *Gliricidia* forage (100-150 g per kg of dietary DM) to untreated and urea-treated straw diets increased milk yields by 14-22% in tropical dairy cattle (Preston and Leng, 1984).

2.7. CONCLUSIONS

a. Fibre, the insoluble substances from the plant cell wall, represents the major fraction of the DM and contains the major energy yielding substrates in low-quality roughages. Its maximum anerobic fermentation is essential for the best utilisation of cereal straws by the ruminant.

b. The digestion of fibre in the rumen is influenced by many interacting factors, including: physico-chemical characteristics of the cell walls, microbial nutrition and host physiology.

c. Digestion kinetic parameters are useful to understand fibre digestion in the rumen and to predict intake and *in vivo* digestibility of forages . Of these parameters, potential degradability has a major effect.

d. Digestion and rate of passage are important factors controlling the utilisation of fibre in the rumen. With low-quality roughages, rate of passage seems to be more important.

e. Particle breakdown and escape from the rumen are the major components of the whole process of fibre passage. Rates of particle breakdown and their influence on intake are still poorly understood. Refinement of the related research techniques and better definition of concepts are required.

f. Because of their chemical composition, nutritive value and fermentation characteristics, highly-digestible fibrous feeds are potentially useful supplements to low-quality roughages.

g. Low levels of supplementation with highly-digestible roughages (0.1-0.2 of the dietary DM) often stimulate intake and digestibility of low-quality roughages. Above these levels, there are high replacement effects irrespective of whether both roughages are fed long and separately or ground in a mixed diet. The resulting increases in digestible DM intake are relatively small.

h. Replacement of low- by high-quality roughages may result from physical limitation of the rumen and sub-optimal supply of nutrients to the microbes.

i. Animal performance can be increased by supplementing with highly-digestible fibrous feeds. Positive associative effects on liveweight gain have been reported, but the factors involved require further investigation.

CHAPTER 3

RUMEN DIGESTION AND INTAKE OF A STRAW-LUCERNE DIET BY STEERS AND WETHERS

3.1. INTRODUCTION

Relative to their energy requirements, cattle obtain more digestible nutrients from low-quality roughages than do sheep (Van Soest, 1982). It is therefore reasonable to focus research on improving the utilisation of cereal straws by cattle. However, experiments with these animals are cumbersome and expensive. Alternatively, sheep can be used. Although results from one species cannot always be directly applied to the other, further knowledge of their differences may help to infer with more confidence. The rôles of rumen fermentation characteristics and diet selectivity in the control of responses to supplementation of straws in diets for sheep and cattle require further investigation (See Section 2.6.1.2).

The dacron bag technique (*in sacco*) is potentially useful to understand the rumen degradation processes and to predict intake (Hovell *et al*, 1986; Ørskov, Hovell and Mould, 1980). Its precision can be improved by controlling factors such as sample size and preparation, sample size/bag area ratio and washing procedures. The technique provides the most useful results when appropriate incubation times and statistical analysis are used (Mertens and Loften, 1980; Nocek, 1985; Ørskov *et al*, 1980).

The objectives of this work were:

- To assess the influence of incubation times on the precision of estimating *in sacco* degradation parameters for the dietary components in wethers fed a straw-lucerne diet.

- To compare the *in sacco* degradation and intake of the dietary components and rumen fermentation in steers and wethers offered a straw-lucerne diet.

3.2. MATERIALS AND METHODS (Experiment 1)

3.2.1. Animals and management

Three Angus x (Friesian x Hereford) steers (711 ±9 kg LW) and nine Suffolk-cross wethers (59 ±4 kg LW) were used. All animals had permanent rumen cannulae; 115 mm diameter in steers and 40 mm in wethers. They were individually housed under continuous illumination. The wethers were harnessed for collection of urine and faeces (McDonald, 1958).

3.2.2. Diets and feeding

Untreated barley straw (*c.v.* Golden Promise) was coarsely shredded in a small -bale tub grinder. It was fed *ad libitum* and supplemented with lucerne pellets at 240 g per kg total dietary DM. This level was selected as being the middle point between the extreme levels of supplementation intended for subsequent trials. The daily allowance of feed was offered in equal portions at 09:00 and 17:00 h. The straw was given after the lucerne pellets were completely consumed. At each feeding, a solution containing 12 g of urea was mixed with the straw (120 ml per kg straw). This was estimated to supply the RDP requirements of the rumen microbes (ARC, 1984) assuming 40 and 100 g CP per kg DM; 6.5 and 9.3 MJ ME per kg DM and CP degradabilities of 0.5 and 0.7 for straw and lucerne, respectively (See Section 2.6.3.2). A premix of vitamins and minerals plus sodium sulphate (Table 3.1A) to attain an RDN:S ratio of about 14:1 (ARC, 1984) was also mixed with the straw. Water was freely available.

During both adjustment and experimental periods straw refusals were collected daily. Refusals for two consecutive days were bulked and dried and the two-day means for straw and total DM intakes calculated. The desired proportion of straw and lucerne in the diet was achieved by adjusting the allowance of lucerne pellets every two days according to the mean straw intake in the preceeding two days. The daily allowance of straw included 0.25 in excess of the straw intake in the previous two days. Straw refusals were sampled daily for further analyses. The experiment consisted of a 14-day adjustment period followed by several periods for incubation of dacron bags and rumen liquour sampling.

3.2.3. *In sacco* degradation of the feeds

Rumen degradation of straw and lucerne was measured using the dacron bag technique as follows:

a. Bag material and making: bags were made from synthetic polyester fabric with 40-50 µ pore size (Sericol Group Ltd, London). Bags measured 9.5 x 24 cm with a round base and had seams double-sewn with nylon thread. The total bag area effectively accessible to rumen microbes was 260 cm^2.

b. Sample size and preparation: A 6 g air-dried sample, ground in a rotary mill fitted with a 2.5-mm screen was used. The effectively accessible bag area/sample size ratio was about 50 cm^2 per g DM.

c. Bag holding device: all bags were tied at the neck with nylon fishing line. In cattle, they were attached along a 90-cm nylon string with a 50-g weight at the bottom end. In sheep, they were attached along a 40 cm plastic-covered wire. This maintained the bags immersed into the rumen content.

d. Incubation procedure: In each of two periods, eight bags containing straw and eight bags containing lucerne were incubated in the rumen of the steers and one bag of each feed removed after 2, 5, 8, 11, 14, 24, 48 and 72 h. As it has been recommended to suspend no more than five bags in the rumen of sheep at a time (Mehrez and Ørskov, 1977), the nine experimental sheep were divided into three groups

according to sets of five incubation times (Table 3.1). Thus, every incubation time considered in the experiment with steers could be included at least once. In each of four periods, five bags containing feed samples were incubated in the rumen and one bag withdrawn at the appropriate times (Table 3.1). In periods 1 and 2, the bags removed at the first, third and fifth incubation times in each group contained straw while those removed at the second and fourth incubation times contained lucerne. The reverse was done in periods 3 and 4. This procedure was adopted in an attempt to reduce effects of the lucerne in the bags on the rumen environment.

e. Bag washing: Immediately after withdrawal, every bag was rinsed and left immersed in cold tap water. Each bag was then washed under the tap while gently manipulating the sample residue, until the effluent was clear.

f. Degradability calculations: The washed bags with residues were dried at 60 °C for 48 h. DM and CP (in lucerne only) of the residues were determined. The *in sacco* results were expressed as g of DM disappearing from the bag per g of DM initially incubated. The water soluble fraction and the particle losses from the bags were determined on four samples of each feed. Bags containing 6 g air-dried samples were soaked in a water bath at 38 °C with continuous circulation for 5 minutes. After removal, each bag was washed as described previously. Losses of particles smaller than 40-50 µ were determined as follows: bags were soaked individually for 5 min in water at 38 °C contained in a beaker (1 l capacity). The bags were then washed as described previously with careful collection of the rinsing water. Particle losses were estimated from the increase in dry weight of a Whatman No. 42 filter paper after filtering the soaking and rinsing waters. These losses were subtracted from the total material disappearing after each soaking time to obtain the water soluble fraction.

3.2.4. Rumen fermentation parameters

On the first and last day of the experimental period, samples of rumen fluid were taken at 0, 1:30, 3:30, 5:30 and 7:30 h post-feeding for pH, NH_3-N and VFA determinations.

3.2.5. Chemical analyses

Unless otherwise specified, the following methods were used in all the experiments of this thesis.

TABLE 3.1 Experimental groups according to *in sacco* incubation times in wethers offered a straw-lucerne diet.

Group[1]	Period	Incubation times, h							
		2	5	8	11	14	24	48	72
1	1,2	S	L	S	-	L	-	S	-
	3,4	L	S	L	-	S	-	L	-
2	1,2	S	-	L	S	-	L	S	-
	3,4	L	-	S	L	-	S	L	-
3	1,2	-	S	-	L	S	L	-	S
	3,4	-	L	-	S	L	S	-	L

1. Three sheep per group.
S=straw; L=lucerne

Dry matter: drying at 100 °C for 24 h in a forced-air oven

Nitrogen: Micro-Kjeldahl

Gross energy: Adiabatic bomb calorimetry

Neutral detergent fibre (NDF): Goering and Van Soest (1970)

Modified acid detergent fibre (MADF): Clancey and Wilson (1966)

Ash: Ashing at 550 °C for 12 h in a furnace

Rumen NH_3-N: Conway microdiffussion analysis as detailed in Appendix 3.2A (Conway, 1962)

Rumen VFA: gas liquid chromatography (g.l.c.) as detailed in Appendix 3.3A. A modification of the method of Erwin, Marco and Emery (1961).

In vitro OMD: two-stage technique of Tilley and Terry (1963).

3.2.6. Statistical analyses

In sacco degradation data for straw and lucerne were analyzed to detect the significance of the incubation period effects. In steers, this was done by analysis of variance (ANOVA) for a nested design with steers as blocks, incubation periods as whole units and incubation times as sub-units (Steel and Torry, 1960). In sheep, the period effect was assessed by ANOVA for a completely randomized design for each incubation time (See Table 3.1). For incubation times of 5, 8, 11 and 14 h, most differences between means of period 1 *v.* 2 and between 3 *v.* 4 were found to be non-significant. Therefore, the data of period 1 and 2 and periods 3 and 4 were pooled, respectively. Means were compared by t-test.

Of the eight times used in wethers, five were to be selected for subsequent *in sacco* studies with sheep fed similar diets since it is preferable to incubate no more than five bags in the rumen of each sheep to facilitate their removal (Mehrez and Ørskov, 1977). A preliminary appraisal of the data showed that a lag time was always greater than 2 h but less than 5 h and therefore the data

for the 2 h incubation could be excluded from further analysis. A non-linear regression was fitted (Modified Newton method of Ross, 1975) to all data after the lag time (McDonald, 1981; Equation 2, Section 2.5.1). The fitted values thus obtained for each incubation time were used as means to generate 50 values per incubation time by a Monte Carlo technique for random numbers generation (Snedecor and Cochran, 1980). This was done for three levels of variation: high, medium and low, corresponding to the minimum, mean and maximum within-incubation time s.d. in the experimental data. Non-linear regressions (Equation 2) were then fitted to the generated values for all incubation times and different combinations of five times, for each level of variation. The two extreme incubation times (5 and 72 h) and a middle point (24 h) were fixed and regressions with several combinations of the remaining incubation times calculated. The criteria to select the most appropriate five incubation times for straw and lucerne were: a) the similarity between the equation parameters with five incubation times and those of the equation with all incubation times; b) the s.e. of these parameters and c) practical considerations such as the time when bags had to be removed and its relation to feeding and ease of withdrawal.

Differences between steers and wethers for *in sacco* degradation of straw and lucerne were assessed by t-test for each incubation time. Digestion kinetic parameters (lag time, potential degradability and degradation rate) were estimated for each animal as suggested by I. McDonald (personal communication). Species differences for kinetic parameters, feed intake and *in vitro* OMD of the straw refusals were compared by t-test.

Rumen pH, NH_3-N and VFA were analyzed by ANOVA for a nested design with animal species as whole units, sampling periods as sub-units and sampling

times as sub-sub-units (Steel and Torry, 1960).

In this and further experiments the GENSTAT statistical computing package was used (Rothamsted Experimental Station, 1983).

3.3. RESULTS

3.3.1. Intake and feed composition

The daily intake of straw was significantly higher ($P<0.01$) in steers than in wethers when expressed as g OM per kg $LW^{0.9}$ (17.1 *v.* 10.9; $s.e._{diff}$ = 2.08) or g OM per kg $LW^{0.75}$ (47.5 *v.* 20.1, $s.e._{diff}$ = 3.96). Straw intakes expressed as g OM per kg LW did not differ ($P<0.05$) between species (9.2 *v.* 7.3, $s.e._{diff}$=1.35).Total OM intakes followed the same trends, as lucerne was always totally consumed. The straw refused by wethers had a significantly lower ($P<0.001$) *in vitro* OMD than that refused by steers (Table 3.2).

The **levels of CP, MADF and minerals in the** straw and lucerne used in this experiment (Table 3.2) were within the range but below the mean values reported in the literature (Tables 2.4 and 2.6). The soluble fraction of straw DM was low (87.4 g/kg) whereas that of lucerne was high (379.8 g/kg). Losses of particles through the dacron bag pores were relatively small (21.9 and 50.9 g/kg DM for straw and lucerne, respectively). As these lost particles may have been degraded as the residues inside the bags, it was considered unnecessary to correct the *in sacco* degradation data for these losses (Hovell *et al*, 1986).

3.3.2. *In sacco* degradation of the feeds

The *in sacco* degradation data for straw and lucerne in steers and wethers are shown in Table 3.3. In steers, there was a significant period effect on the *in sacco* degradation of straw DM. However, the differences between means were significant ($P<0.05$) only at 14 h of incubation. In wethers, differences

TABLE 3.2 Chemical composition and *in vitro* OMD of straw and lucerne as offered to steers and wethers.

	Straw	Lucerne
DM composition, g/kg		
CP	26	177
Ash	33	109
NDF	810	409
MADF	463	272
Ca	3.1	14.4
P	0.7	2.1
Mg	0.6	1.6
Na	0.7	3.9
In vitro OMD[1], g/g		
As fed	0.533	0.887
Refusals		
Steers	0.513	-
Wethers	0.452	-
s.e. diff	0.007	-

1. Corrected by linear regression of *in vivo* OMD on *in vitro* OMD of standard samples.

TABLE 3.3 Means and incubation-period effects for *in sacco* degradation of straw DM and lucerne DM (g/g) in the rumen of steers and wethers offered a straw-lucerne diet.

Feed and species	Incubation period	Incubation times, h						
		5	8	11	14	24	48	72
Straw								
Steers[1]	1	0.097	0.148	0.185	0.230	0.330	0.486	0.533
	2	0.117	0.173	0.214	0.276	0.338	0.514	0.554
		NS	NS	NS	*	NS	NS	NS
Wethers	1 + 2	0.088	0.121	0.224	0.237	0.421	0.485	0.537
	3 + 4	0.108	0.184	0.228	0.254	0.411	0.523	0.585
	s.e. diff	0.003	0.028	0.024	0.019	0.026	0.023	0.017
		**	NS	NS	NS	NS	NS	NS
Lucerne								
Steers[1]	1	0.443	0.513	0.532	0.598	0.682	0.769	0.784
	2	0.445	0.480	0.564	0.617	0.682	0.755	0.776
		NS	NS	NS	NS	NS	NS	NS
Wethers	1 + 2	0.450	0.555	0.669	0.651	0.704	0.752	0.760
	3 + 4	0.440	0.565	0.589	0.612	0.697	0.741	0.766
	s.e. diff	0.011	0.043	0.032	0.037	0.014	0.018	0.015
		NS	NS	NS	NS	NS	NS	NS

1. Overall s.e. of the difference for period means within the same incubation time: straw = 0.015; lucerne = 0.020 (See Table 3.4A).

NS = Not significant; * $P<0.05$; ** $P<0.01$

between means of periods 1+2 and those of periods 3+4 were non-significant, except at 5 h. Period differences for *in sacco* degadation of lucerne DM were non-significant in both steers and wethers. Lucerne CP and DM degradation were positively correlated ($P<0.01$). The linear regressions of *in sacco* degradation of lucerne CP (Y) on lucerne DM (X) with data pooled across periods and incubation times were:

$Y = -0.015 + 1.18X$ r = 0.99 r.s.d. = 0.021; in steers
$Y = 0.017 + 1.15X$ r = 0.98 r.s.d. = 0.025; in wethers

Further analyses designed to permit selection of the most appropriate incubation times and to compare *in sacco* degradation of feeds in the two species were done with data pooled across incubation periods.

3.3.3. Selection of five incubation times

There were no major differences or clear trends in the size of the parameters and their s.e. for equations with different combinations of five incubation times compared with equations with all incubation times. This was much the same for the low, medium and high levels of variation and results for the medium level only are given (Table 3.4). For straw DM, the equation with 5, 8, 14, 24 and 72 h gave values for the parameters which were virtually identical to those of the equation with all the times. The variation was slightly larger for lucerne DM, but again the differences between the parameters and their s.e. were small and inconsistent. For lucerne, the equation with 5, 11, 14, 24 and 72 h gave values for the parameters which were closest to the values of the equation with all the times.

TABLE 3.4 Parameter estimates and their s.e. for the equation $Y = a + B(1-e^{kt})$ fitted to different combinations of incubation times (t,h) and *in sacco* degradation of straw and lucerne DM (Y, g/g) generated by random numbers from experimental results obtained with wethers offered a straw-lucerne diet.

Feed and incubation times	Equation parameters and s.e.					
	a	s.e.	B(x10^3)	s.e.	k(x10^3)	s.e.
Straw						
5 8 11 14 24 48 72	-51	8.6	609	7.5	55.2	1.8
5 8 11 - 24 - 72	-54	9.0	611	8.5	56.3	2.0
5 8 - 14 24 - 72	-51	8.6	609	8.3	55.2	1.9
5 8 - - 24 48 72	-54	8.9	611	7.5	56.6	2.2
5 - 11 14 24 - 72	-52	9.3	610	9.0	55.2	1.9
5 - 11 - 24 48 72	-54	9.3	611	8.1	56.3	2.0
5 - - 14 24 48 72	-52	9.1	610	8.2	55.2	1.9
Lucerne						
5 8 11 14 24 48 72	225	19.6	520	18.6	121.1	5.7
5 8 11 - 24 - 72	218	22.9	529	21.4	122.9	7.1
5 8 - 14 24 - 72	238	20.4	510	18.8	115.2	6.7
5 8 - - 24 48 72	231	25.9	515	24.6	118.5	8.6
5 - 11 14 24 - 72	228	20.2	520	18.8	118.7	6.3
5 - 11 - 24 48 72	216	22.7	529	21.6	123.7	7.1
5 - - 14 24 48 72	236	19.6	511	18.4	114.7	6.3

3.3.4. Steers *versus* wethers

Species differences for *in sacco* degradation of straw DM were non-significant at all incubation times, except at 24 h (Table 3.5); with no general trend across times. Lucerne DM degradation tended to be higher in wethers than in steers up to 24 h. At longer incubation times, lucerne DM degradation was higher in steers than in wethers, though this was significant only at 72 h.

Digestion kinetic parameters for straw and lucerne were calculated from non-linear equations fitted to the data of each steer and of each wether in group 3 (See Table 3.1). Data for this group of sheep were selected because three determinant points to fit the non-linear regressions, *i.e.* 5, 24 and 72 h were included. The same incubation times were used to derive equations for the steer data. The results for digestion kinetic parameters for straw and lucerne are shown in Figures 3.1 and 3.2, respectively. Lag times and potential degradabilities for straw DM were similar in both species while the degradation rate was faster in wethers than in steers ($P<0.05$). Lag times for lucerne DM were not significantly different between species ($P<0.05$). The potential degradability for lucerne DM was higher and the degradation rate slower ($P<0.05$) in steers than in wethers.

3.3.5. Rumen fermentation parameters

ANOVAs for rumen pH, NH_3-N and VFA were conducted with all steers and the three sheep in group 3 only.The results are given in Figure 3.3 and a summary of the ANOVA is given in Table 3.5A of the Appendix. Rumen pH varied within a narrow range (6.4-7.3). Although the species and period effects were non-significant ($P<0.05$) the pH means were always higher in wethers than in steers. Generally, rumen NH_3-N was lower and reached a peak later after feeding in steers than in wethers. The NH_3-N values were variable with mean

TABLE 3.5 *In sacco* degradation (g/g) of straw and lucerne in the rumen of steers and wethers fed a straw-lucerne diet.

Incubation times, h	Straw DM			Lucerne DM		
	Steers[1]	Wethers[2]	s.e. diff	Steers[1]	Wethers[2]	s.e. diff
5	0.107	0.098	0.007	0.444	0.445	0.007
8	0.160	0.152	0.020	0.497[c]	0.560[d]	0.027
11	0.199	0.226	0.014	0.548[c]	0.629[d]	0.025
14	0.253	0.246	0.017	0.607	0.632	0.024
24	0.334[a]	0.416[b]	0.020	0.682	0.700	0.012
48	0.500	0.504	0.019	0.762	0.746	0.016
72	0.544	0.561	0.014	0.780[c]	0.763[d]	0.007

1. Means of 6 observations per incubation time.
2. Means of 12 observations for 5, 8, 11, 14, 24 and 48 h and of 6 observations for 72 h.
   Means in the same row, within feed, showing different superscripts are significantly different ($P<0.05$).

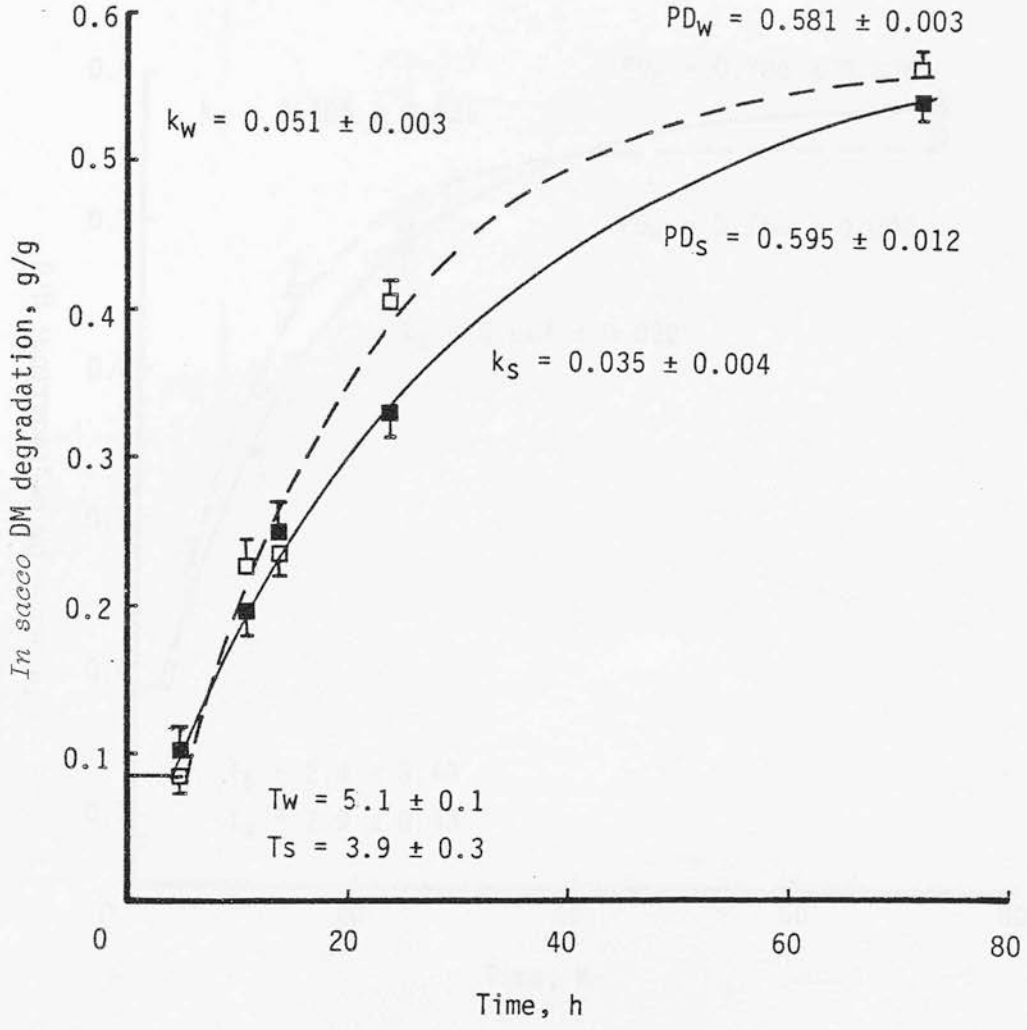

FIGURE 3.1 *In sacco* degradation curves and kinetic parameters for straw in steers (S, ——) and wethers (w, ---) offered a straw-lucerne diet.

T = lag time, h; k = degradation rate, per h;
PD = potential degradability, g/g. ± indicates s.e.

(Non-linear regressions calculated using six values for each incubation time; the symbols indicate means for each incubation time and the length of vertical bars the s.e. mean).

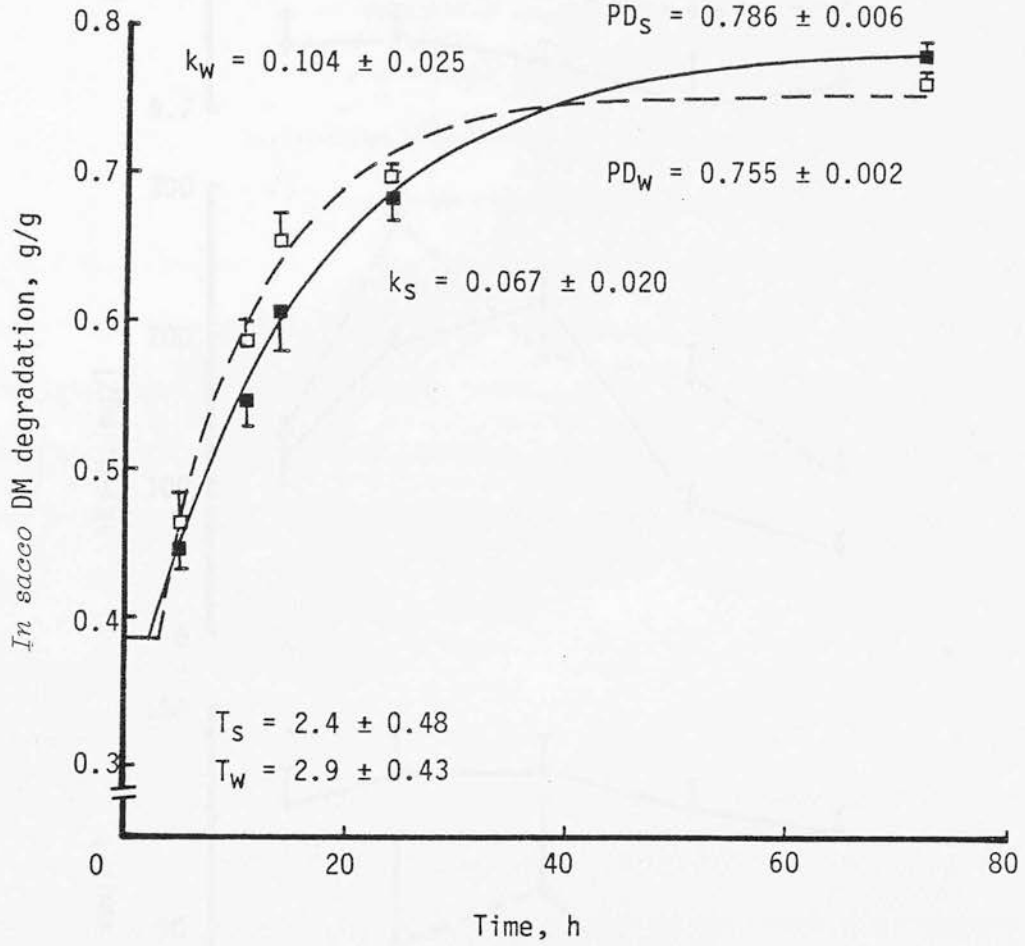

FIGURE 3.2 *In sacco* degradation curves and Kinetic parameters for lucerne in steers (s, ——) and wethers (w ---) offered a straw-lucerne diet.
T = lag time, h; k = degradation rate, per h;
PD = potential degradability, g/g. ± indicates s.e.
(Non-linear regressions calculated using six values for each incubation time; the symbols indicate means for each incubation time and the length of vertical bars the s.e. mean).

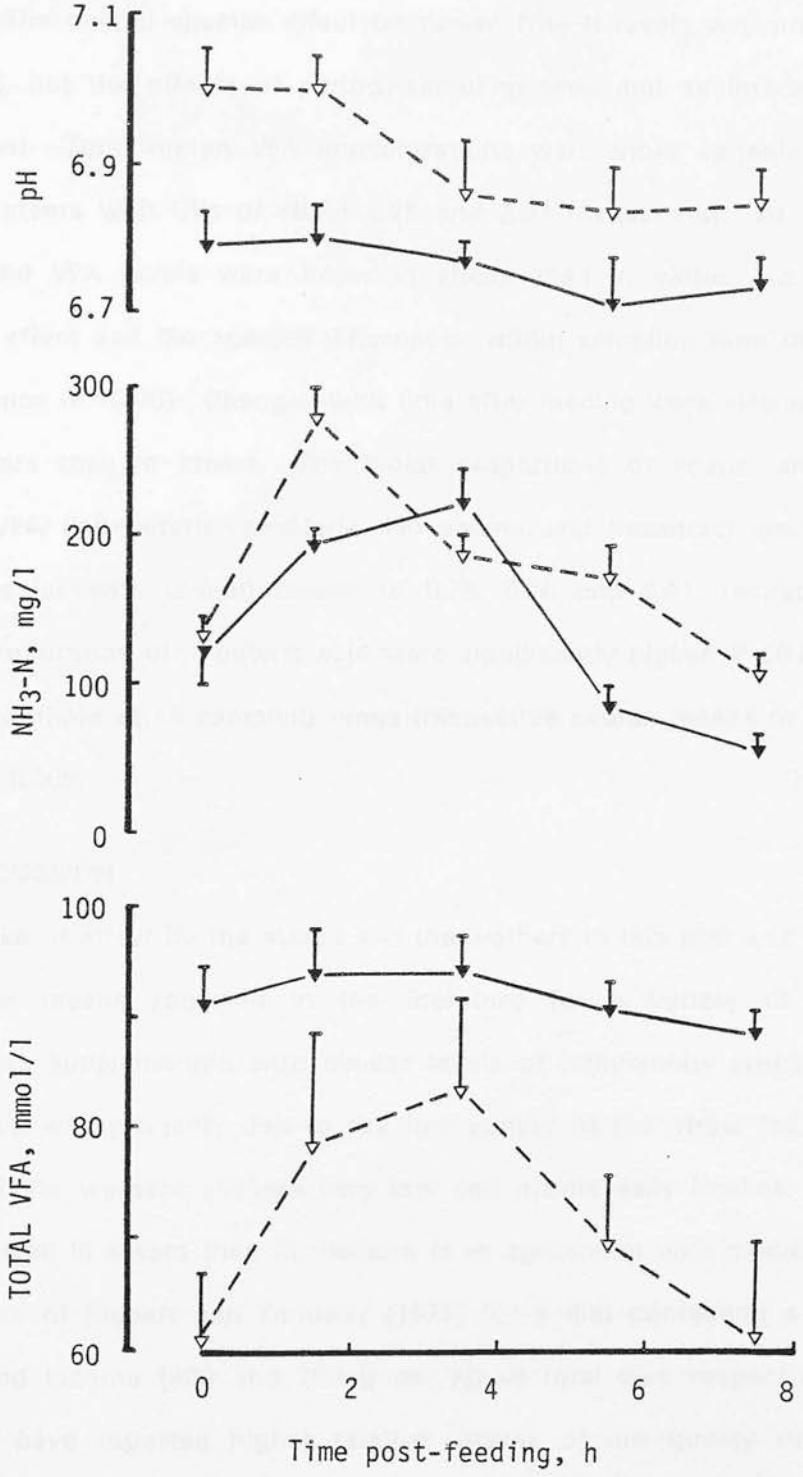

FIGURE 3.3 Changes in rumen pH and levels of NH_3-N and VFA with time post-feeding in steers (▼—▼) and wethers (▽---▽) offered a straw-lucerne diet. (The length of vertical bars indicates s.e. means).

CV within sampling time and period of about 0.20 in wethers and 0.25 in steers. The overall species effect on rumen NH_3-N levels was not significant ($P<0.05$), but the effects of period, sampling time and all interactions were significant. Total rumen VFA concentrations were more variable in wethers than in steers with CVs of about 0.25 and 0.09 respectively. At all sampling times, the VFA levels were lower in sheep than in cattle, but the overall species effect and the species differences within sampling time did not reach significance ($P<0.05$). Changes with time after feeding were also more marked in wethers than in steers. The molar proportions of acetic, propionic and higher VFA (iso-butyric, n-valeric, iso-valeric and hexanoic) were similar for both species with overall means of 0.76, 0.16 and 0.01, respectively. The molar proportions of n-butyric acid were significantly higher ($P<0.05$) in steers than in wethers at all sampling times (respective overall means of 0.08 *v.* 0.05, $s.e._{diff} = 0.005$).

3.4. DISCUSSION

The intake of straw by the steers and the wethers in this trial was much lower than the means reported in the literature for a variety of low-quality roughages supplemented with similar levels of leguminous crops (See Table 2.8). This was probably due to the low quality of the straw fed. Moreover, some of the wethers showed very low and erratic daily intakes. The higher relative OMI in steers than in wethers is in agreement with calculated results from data of Siebert and Kennedy (1972) for a diet containing a low-quality grass and lucerne (800 and 200 g per kg of total diet, respectively). Other workers have reported higher relative intakes of low-quality roughages by cattle than by sheep when expressed as g per kg $LW^{0.75}$ (Blaxter, Wainman and Davidson, 1966) or as g per kg $LW^{0.92}$ (Bird, 1974). However, the present results are at variance with those of Playne (1978a) (g/kg $LW^{0.9}$) and Prigge *et*

al (1984) (g/kg $LW^{0.75}$), who found no species differences in intake of mature hays and also with the results of Rees and Little (1980), who found higher intakes of mature grasses (g/kg $LW^{0.9}$) in sheep than in cattle. The age and weight of the animals, the physical form of the forage, the feeding regime and level (*i.e.* the amount of refusals allowed) and other experimental factors differ quite widely between the present trial and those mentioned. These factors are likely to affect the results. For example; a) within a particular experiment, species differences in forage intake can vary according to animal weight and age (Greenhalgh and Reid, 1973); b) by increasing the amount allowed for refusals, the intake of straw has been shown to increase significantly (Wahed and Owen, 1986b). The conditions under which the animals were kept in the present trial may have influenced the results . For reasons of hygiene in the crates, the sheep were harnessed for urine and faeces collection whereas the steers were kept in pens tied by their necks. Greenhalgh and Reid (1973) observed that for both sheep and cattle , the intake of roughages was lower in the animals harnessed for collection of faeces than in their unharnessed counterparts.

The lower *in vitro* OMD of the straw refused by wethers compared to that by steers indicates that the former selected a better quality diet. Prigge *et al* (1984) reported non-significant differences in the composition of the forage consumed by steers and wethers. The straw in the present trial was of lower quality than their hay, which was hammer-milled through a 4-cm screen and fed *ad libitum* with a 0.15 refusal rate. These factors may have reduced the opportunity for diet selection by sheep. Although the differences did not reach statistical significance in the work of Playne (1978a), sheep ate a diet with less fibre and lignin than cattle. They offered a poorer quality hay at a higher refusal rate (0.4-0.5) than did Prigge *et al* (1984). The results with

sheep in the present trial confirm those of Wahed and Owen (1986a) regarding the lower *in vitro* OMD of the refusals when cereal straws are fed long and *ad libitum* with about 0.20 excess over previously observed intakes.

The similarity between steers and wethers in their *in sacco* degradation of straw DM at most incubation times is in line with the results of Prigge *et al* (1984) for a mature grass. Despite the high variation among animals, the degradation rate of straw DM was significantly higher in wethers than in steers. This result could have been partly due to the fact that the degradation of straw at 24 h was much higher in wethers than in steers; this difference was larger than that at any other incubation time. The 24-h point had a strong influence on the degradation rate estimated by non-linear regression analysis.

The faster degradation rate and higher *in sacco* degradation of lucerne DM up to 24 h in wethers than in steers could be associated with differences in the rumen microflora. The more easily fermentable fractions of the straw selected by the wethers may have stimulated the activity of their rumen microbes enabling them to degrade the more digestible lucerne fractions at a higher rate. The more refractory fractions were degraded better in the rumen of steers as indicated by higher degradations at 48 and 72 h. Similarly, a faster rate of degradation but with a tendency for a lower potential degradability in wethers than in steers was observed for straw DM. When fed the same roughage, sheep had shorter mean digesta retention times than did cattle (Poppi *et al*, 1981b; Prigge *et al*, 1984; Rees and Little, 1980; Uden, Rounsaville, Wiggans and Van Soest, 1982). Microorganisms in the rumen of sheep may have adapted to shorter retention times by accelerating the degradation rate of the feeds. Such a mechanism was apparent in the results of Poppi *et al*

(1981b) and Hendricksen, Poppi and Minson (1981) for the stem and leaf fractions of a mature grass and a legume forage. The NDF degradation rates were faster while the NDF mean retention times in the rumen were shorter in sheep than in cattle. In these and other reports (Mertens and Ely, 1982; Playne, 1978b; Prigge *et al*, 1984; Rees and Little, 1980) the *in vivo* digestibility of low-quality roughages was higher in cattle than in sheep. *In vivo* digestibilities were not determined in the present trial. However, the effective degradability of the feeds in the rumen calculated as proposed by McDonald (1981), should illustrate species differences in the extent of degradation in this organ, where at least 0.6-0.7 of the total OM digested is fermented (Egan *et al*, 1975). The effective degradability is calculated taking into account the digestion kinetic parameters (digestion rate and lag phase) and the outflow rate of digestion from the rumen (*i.e.* the reciprocal of mean retention time). The mean retention time (MRT) of mature forages in the rumen of cattle is about 1.35 times that in sheep (Poppi *et al*, 1981b; Prigge *et al*, 1984; Rees and Little, 1980; Uden *et al*, 1982). Assuming a rumen MRT of straw of 50-60 h in cattle and 37-44 h in sheep and using the equation parameters shown in Figure 3.1, it can be calculated that the effective degradabilities of straw DM in the rumen of steers and wethers are about 0.40 and 0.38 . These results and the species differences in relative intakes observed in the present trial indicate that a higher relative intake of total digestible nutrients could have been achieved by the steers as compared to the wethers. However, this does not take into account the selection of the more digestible straw fractions by wethers which would tend to offset this advantage.

Significant correlations between lucerne DM and CP degradation in both steers and wethers were found. Such relationships can be used to predict CP degradability using the more easily determinable DM degradability. Data on a

wider range of diets should, however, be analyzed to assess whether the relations remain similar. Ahmed (1982) and Nocek, Cummins and Poland (1979) also observed high correlations between *in sacco* degradation of DM and CP in a variety of feeds.

The values for NH_3-N and VFA in the rumen were very variable and therefore the results should be treated with caution. After feeding rumen NH_3-N tended to be higher and reached a peak faster in wethers than in steers. This may have been a result of a faster degradation of the lucerne CP and/or the urea, and the selection by sheep of straw fractions with higher CP. Prigge *et al* (1984) also reported higher rumen NH_3-N in sheep than in cattle fed a mature grass at high and low levels of intake. The higher variation in total concentrations of rumen VFA with time after feeding in sheep could also reflect the degradation of a diet containing plant fractions of faster fermentation rates. Higher and steadier levels of VFA were maintained in the rumen of steers which had relatively higher intakes than did sheep. In the work of Prigge *et al* (1984), higher levels of intake were associated with higher total VFA in the rumen of sheep and cattle.

The results regarding the selection of the five incubation times required for further *in sacco* degradation studies indicated that when determinant points in the degradation-time curves are selected, other incubation times exert relatively small effects on the outcome of fitting non-linear regressions. However, with the large influence of 24 h it would seem that other incubation times around 24 h are important. For straw and lucerne DM, the 72-h degradation was about 0.90-0.95 of the asymptote of the curve, indicating that little would be gained with data obtained beyond this time. Lag time was always less than 5 h but close enough to 5 h to be safely taken as a starting

point. A sharp inflexion was observed between 20 and 40 h, which justified the selection of the 24-h incubation. The 14-h time was not selected because of the inconvenience of sampling during the night. Incubation for 8 h was avoided as bag removal often interfered with the afternoon feeding . Taking all these factors into account the five incubation times selected for further *in sacco* studies were: 5, 11, 24, 48 and 72 h.

3.5. CONCLUSIONS

a. When long straw is fed *ad libitum*, sheep select the more digestible plant fractions. Their relative intake is lower than that of cattle (for liveweights similar to those in the present trial).

b. Straw and lucerne tend to be degraded faster but to a lesser extent in the rumen of sheep than in the rumen of cattle.

c. Of the times studied in this experiment, the most appropriate times for incubation of straw and lucerne in the rumen of sheep to estimate *in sacco* degradation parameters are 5, 11, 24, 48 and 72 h.

CHAPTER 4

SUPPLEMENTATION OF BARLEY STRAWS WITH LUCERNE IN DIETS FOR SHEEP

4.1. INTRODUCTION

Leguminous crops are potentially valuable supplements for low-quality roughages in diets for ruminants. They contain high levels of protein, energy and other nutrients and promote higher microbial growth in the rumen than do most other feeds fed to sheep (ARC, 1984). The optimum outcome of supplementing low-quality roughages with these crops will be to maintain or increase the intake and digestion of the roughage thereby increasing the total intake of digestible nutrients. Low levels of supplementation with long or chopped leguminous hays (0.1-0.2 of the dietary DM) have often stimulated the intake and digestion of low-quality roughages. Above these levels, high replacement effects and hence low increases in the total intake of digestible nutrients have been observed (See Section 2.6.3.2). This may have been due to a physical limitation of the rumen and/or an insufficient nutrient supply to the rumen microbes. If these two constraints could be overcome, a better use of both the leguminous crop and the low-quality roughage may be achieved.

The objective of this work was to study the effect of supplementing barley straws with lucerne pellets and/or urea on the *in sacco* degradation, the *in vivo* digestion and the intake of the dietary components. Some fermentation parameters in the rumen of sheep fed the different rations were also studied.

4.2. MATERIALS AND METHODS

Four experiments were carried out. Experiments 2 and 4 were *in sacco* degradation studies with untreated and ammonia-treated straw, respectively. Experiments 3 and 5 were the corresponding *in vivo* digestion studies. For

these and further trials, two batches of barley straw (*c.v.* Golden Promise) and lucerne were purchased. Their chemical composition and use are detailed in Table 4.1. A different batch was used in Experiment 1 only (Table 3.2).

4.2.1. Supplementation of untreated straw with lucerne

4.2.1.1. Experiment 2: *In sacco* degradation of the feeds and rumen fermentation

a. Animals and management

Eleven Suffolk-cross wethers (58.6 ±1.8 kg LW) fitted with permanent rumen cannulae (40 mm diameter) were used. They were harnessed for separate collection of urine and faeces (McDonald, 1958) and kept in individual crates under continuous illumination.

b. Diets and feeding

Untreated barley straw (*c.v.* Golden Promise) was coarsely shredded in a small-bale tub grinder. It was offered *ad libitum* and supplemented with lucerne pellets at the following levels (g per kg total diet, DM basis): 0 (US-0L), 160 (US-16L), 320 (US-32L) and 480 (US-48L). A fifth diet consisted of lucerne pellets alone (100L), fed at about 0.8 of the *ad libitum* intake. The daily allowance of feed was offered in equal amounts at 09:00 and 17:00 h. The straw was offered after the lucerne pellets were completely consumed. At each feeding, a solution containing the necessary amount of urea to supply the RDP requirements of the rumen microbes (ARC, 1984) was thoroughly mixed with the straw (300 ml per kg straw). These requirements were calculated using the assumed values for CP, ME and CP degradabilities for straw and lucerne given for Experiment 1. A premix of vitamins and minerals containing sodium sulphate (Table 3.1A) to attain a RDN: S ratio of 14: 1 (ARC,

TABLE 4.1 Average chemical composition of the straw and lucerne batches used in the experiments.

	Composition of the DM					Use in experiments no.		
	g/kg				MJ/kg	*In sacco*[2]		*In vivo*[2]
	CP	Ash	NDF	MADF	GE	Incubated	Fed	Fed
Straw								
Batch 1								
Untreated	29	48	832	503	18.4	2,7	2	3
Batch 2								
Untreated[1]	31	49	841	515	18.8	-	7	8
Ammonia-treated[1]	96	51	793	524	18.8	4,9	4,9	5,10
Lucerne								
Batch 1[1]	170	105	437	292	18.4	2,4	2,4	3,5
Batch 2	167	109	489	311	18.4	7,9	7,9	8,10,11

1. These batches were used in Experiment 6, Chapter 5.
2. Experiments 7 to 11 are described in Chapter 6

1980) was also mixed with the straw. Water was freely available.

Straw refusals were collected daily. Refusals for two consecutive days were bulked and dried and the two-day means for straw and total DM intake calculated. The desired proportion of straw and lucerne in the diets was achieved by adjusting the allowance of lucerne pellets every two days according to the previous mean straw intake. The straw allowance was adjusted to include 0.25 excess over this intake.

The experiment was divided in two parts. In Part 1, diets US-0L, US-16L and US-32L were offered to 3 sheep per diet. In Part 2, the same three sheep were offered diet US-32L and six other sheep were randomly allocated to diets US-48L and 100L. Two sheep used in Part 1 finished in poor condition and were replaced in Part 2. In each part, the experimental period consisted of 14 days for adjustment followed by four periods of dacron bag incubations separated by 48-h rest periods. Each period corresponded to the incubation of five bags. Straw was incubated in periods 1 and 2 and lucerne in periods 3 and 4. Rumen pH, NH_3-N and VFA were analyzed in samples withdrawn at 0, 1:30, 3:30, 5:30 and 7:30 h post-feeding on the day before and after this series of incubation periods.

c. *In sacco* degradation of the feeds

Rumen degradation of straw and lucerne was measured using the dacron bag technique as outlined for Experiment 1, except that only five incubation times (5, 11, 24, 48 and 72 h) were considered on each period and all bags contained the same feed. *In sacco* degradation of DM and NDF in straw and DM and CP in lucerne were measured. Straw was not incubated in the rumen of sheep receiving pellets alone and lucerne was not incubated in the rumen of sheep receiving straw alone. The water soluble fraction and the losses of particles

through the dacron bag pores were determined for straw as in Experiment 1.

d. Rumen outflow rate of lucerne particles

The outflow rate of insoluble particles of lucerne from the rumen was determined with chromium(Cr)-mordanted material. This was prepared as described by Ganev, Ørskov and Smart (1979), except that the lucerne pellets were previously washed with warm water to remove all soluble matter and the final dried material was broken manually. Washing was done by repeatedly mixing the Cr-lucerne with tap water in a large container and decanting off the water after the solids had settled. Before the Cr-lucerne was used, several tests were conducted to assess the effectiveness of the mordanting technique and its effect on particle size distribution. Total losses of Cr-lucerne DM from dacron bags incubated in the rumen of sheep or suspended in a water bath were estimated (Eliman and Ørskov, 1984b). Six bags, each containing 6 g of the Cr-lucerne were immersed in a bath with circulating water at 38 °C for 12 h while 2 bags were incubated for 12 h in the rumen of each of three sheep fed untreated straw and lucerne. The losses of Cr-lucerne DM, after correcting for losses of small particles through the bag pores were: 235.5 ± 16.7 and 192.5 ± 5.4 g per kg for bags incubated in the rumen and immersed in the water bath, respectively. The Cr concentration in the original material was higher (72 g Cr per kg DM) than in the residues after incubation in the rumen or in the water bath (65.6 and 66.4 g Cr per kg DM, respectively). This indicated that with the method of washing used some soluble lucerne DM and unbound Cr were not removed. No further DM losses or reductions in the Cr concentration of the residue were found after 12 h of incubation. It was therefore considered that the Cr-lucerne material remaining after 12 h of

incubation was rendered insoluble and indigestible. The particle size distribution of this material was measured by wet sieving of 10-g DM samples for 20 min in a test sieve shaker (Model EVS1, Endecotts Ltd, London) at a vibration amplitude of about 3 mm. Lucerne pellets and Cr-lucerne were soaked in tap water overnight. Cr-mordanting increased the proportion of fine particles in the lucerne as compared to the original lucerne pellets. Respective proportions of DM retained (g per kg total DM retained) on sieves with aperture size of 0.6 mm (side of a square hole); passing a 0.6 mm-but retained on a 0.15-mm sieve and passing a 0.15 mm-sieve were 347, 333 and 320 for the pellets and 106, 377 and 517 fo the Cr-lucerne. It was considered that such a change in particle size would not have resulted in major errors if the Cr-mordanted lucerne was assumed to represent the lucerne particles in the rumen. These particles in the rumen would have been smaller than those in the original pellets due to breakdown through mastication.

A tissue-paper pellet containing 50 g of the prepared Cr-lucerne was immersed into the rumen of two sheep on each of the following diets: US-16L, US-32L, US-48L and 100L. Total faeces collections were done after 12, 16, 20, 24, 28, 32, 48, 60, 72 ,96 and 120 h. The Cr concentration in all faecal samples was determined . For each sheep, a linear regression was fitted to data in the descending portion of the plot of the natural logarithm of Cr-concentration in faeces against time. The slope of this regression was interpreted as the outflow rate (k_f) of undigested lucerne particles from the rumen (Eliman and Ørskov, 1984a).

4.2.1.2. Experiment 3: Intake and *in vivo* digestibility of diets containing untreated straw and lucerne

a. Animals and management

Sixteen Suffolk-cross wethers of 32.5 ±0.6 kg LW were used. They were harnessed for collection of faeces and kept in individual crates under continuous lighting. The crates were designed for direct urine collection.

b. Diets and feeding

The diets and feeding management were as in Experiment 2, except that diet 100L was not included and the experiment was done in one part. The experiment comprised 10 days when urea-supplemented straw was fed to all sheep, a 14-day period for adjustment to the straw-lucerne diets and a 13-day period for intake measurements and collection of faeces and urine.

c. *In vivo* digestibility of the diets

The *in vivo* digestibility of DM, OM, NDF, MADF, CP and GE in the diets was determined by standard procedures (Schneider and Flatt, 1975). The ME concentration of the diets was calculated using predicted values for methane (Blaxter and Clapperton, 1965). Urine and faeces were collected daily for 10 days and bulked. Faeces were stored frozen while urine was stored at room temperature. The urine pH was adjusted daily to 3.5 by addition of 0.25 (v/v) H_2SO_4. Urine-collecting funnels were rinsed twice daily with 0.02 (v/v) H_2SO_4.

4.2.2. Supplementation of ammonia-treated straw with lucerne

4.2.2.1. Experiment 4: *In sacco* degradation of the feeds and rumen fermentation

a. Animals and management

Twelve Suffolk-cross wethers of 60.0 ±8.7 kg LW were used. They were fistulated, harnessed and housed as in Experiment 2.

b. Diets and feeding

Ammonia-treated barley straw (*c.v.* Golden Promise) was used. It was treated in an oven (Fma Processing Plant, Fma & Co, England) with anhydrous ammonia at about 35 g NH_3 per kg of straw DM for 23 h, with a peak temperature of 90 °C. The straw was fed *ad libitum* and supplemented with lucerne pellets at the following levels (g per kg total diet, DM basis): 0 (TS), 160 (TS-16L), 320 (TS-32L) and 480 (TS-48L). Other feeding procedures were as in Experiment 2, except that urea was not used. Rumen degradabilities of the CP in the original untreated straw and of the CP added through ammoniation were assumed to be 0.5 and 0.7, respectively (Abidin and Kempton, 1981; Dryden and Kempton, 1983; Solaiman, Horn and Owens, 1979).

Experimental periods for adjustments, dacron bag incubations and rumen sampling were as for Part 1 in Experiment 2, except that rumen fluid samples were taken at 0, 2, 4 and 7 h post-feeding.

c. *In sacco* degradation of the feeds

The *in sacco* degradation of straw and lucerne was determined as described for Experiment 2, except that the bag holding device was replaced. This was a modification of a design from the Animal and Grassland Research Institute, Hurley (A.P. Williams, personal communication). It consisted of a semirigid

plastic tube (30 cm long, 12-mm outside diameter) with five equally spaced slots. The necks of the bags were inserted through the slots and folded over their lower portion; both being secured together with a rubber ring. The water soluble fraction and the particle losses through the dacron bags pores for ammonia-treated straw were determined as in Experiment 1.

d. Rumen outflow rate of lucerne particles

This was estimated as in Experiment 2, except that faecal collections were done at 24, 28, 32, 48, 60, 72, 96 and 120 h after dosing the sheep on all treatments containing lucerne.

4.2.2.2. Experiment 5: Intake and *in vivo* digestibility of diets containing treated straw and lucerne

a. Animals and management

Sixteen Suffolk-cross wethers of 39.1 ±0.4 kg LW were used. They were harnessed, crated and housed as in Experiment 3.

b. Diets and feeding

The sheep were fed the same diets used in Experiment 4. The experiment consisted of 10 days when ammonia-treated straw alone was fed to all sheep, a 14-day period for adjustment to the straw-lucerne diets and a 13-day period for intake measurements and collection of faeces and urine.

c. *In vivo* digestibility of the diets

The same procedures described for Experiment 3 were used.

4.2.3. Chemical analyses

In all experiments the analyses of feed, faeces and urine were done as described in Chapter 3. Chromium in faecal samples was determined by

atomic absorption spectrophotometry as detailed in Appendix 4.1A.

4.2.4. Statistical analyses

The dietary treatments in the *in sacco* degradation studies with untreated (Experiment 2) and treated (Experiment 4) straw were arranged in a completely randomized block design with three sheep per diet and blocks according to LW at the beginning of the experiment. *In sacco* degradation data for straw and lucerne were analyzed by ANOVA for a nested type design with three strata: diets as whole-units, incubation periods as sub-units and incubation times as sub-sub-units (Snedecor and Cochran, 1980; Steel and Torry, 1960). The diet effect was partitioned into its linear and quadratic components. Following the recommendations of Steel and Torry (1960) for nested designs in time, the pooled residual sum of squares of each stratum was initially partitioned into the respective block interactions . The effects of diet, incubation period and incubation time were tested against the respective block interactions in the residual mean squares or against the pooled residual mean square of the stratum. If these tests gave similar results, the pooled residual mean square remained in the model of the design. A similar statistical analysis was conducted for pH and NH_3-N in the rumen, except that diets were the whole units, sampling days the sub-units and sampling times the sub-sub-units. VFA determinations were done on samples obtained by mixing equal volumes of rumen fluid from each of two sampling days. Therefore, the corresponding statistical analysis was done as for a nested design with two strata: diets as whole units and sampling times as sub-units.

In experiment 2, with untreated straw supplemented with lucerne, the effect of the experimental part was assessed for intake and the *in sacco* degradation of straw and lucerne before the complete analysis with all five diets was done.

As mentioned previously, the experiment was divided into two parts. Diets US-0L, US-16L and US-32L were offered to the sheep in Part 1 whereas diets US-32L, US-48L and 100L were offered in Part 2. The same sheep received diet US-32L in both experimental parts. Part effects on straw and total OMI were tested by ANOVA for a completely randomized design removing the sheep effect from the residual. The *in sacco* degradation data were analyzed as for a nested type design where sheep were the blocks, parts the whole-units, incubation periodsthe sub-units and incubation times the sub-sub-units.

In Experiments 2 and 4 non-linear regressions (Equation 2, Section 2.5.1) were fitted to the *in sacco* degradation data for straw and lucerne for each sheep within or across periods, depending on the significance of the period effect. Digestion kinetic parameters (degradation rate , potential degradability and lag time) were then calculated according to the model of McDonald (1981) (See Section 2.5). The same model was used to calculate the effective degradability of lucerne (dg) as follows:

$$dg = A + [(B \times k)/(kf + k) \times e^{-(kf + k)xT} \qquad \text{(Equation 6)}$$

where:

dg = effective degradability
A = soluble fraction
B = non-linear regression constant
k = degradation rate constant
kf = fractional outflow rate of insoluble particles from the rumen
T = lag time

As fractional outflow rates were determined only in two sheep per diet, each treatment mean was used to estimate the degradabilities for each sheep within diet. Diet effects for digestion kinetic parameters, outflow rate of lucerne particles from the rumen, lucerne effective degradability and mean daily intake in the *in sacco* degradation studies (Experiments 2 and 4) were

tested by ANOVA for a completely randomized block design. Intake data for lucerne pellets alone were excluded from the analysis. In addition, linear regressions of total OMI on lucerne OMI and straw OMI on lucerne OMI were calculated using the data from all diets.

The significance of the diet effect (level of lucerne) on the *in vivo* digestibility and intake of diets based on untreated (Experiment 3) or treated (Experiment 5) straw was tested by ANOVA for a completely randomized block design with four replicates per treatment. The diet effect was partitioned into its linear and quadratic components. The sheep were blocked according to their straw intake during the first ten days of the experiment, when the straws were fed alone. Linear regressions of lucerne OMI on total OMI, straw OMI or total DOMI were calculated using the data from all diets in Experiments 3 and 5.

4.3. RESULTS

4.3.1 Supplementation of untreated straw with lucerne

4.3.1.1. Experiment 2: *In sacco* degradation of the feeds and rumen fermentation

a. Intake and *in sacco* degradation of the feeds

The means for straw and total OMI by the sheep offered diet US-32L were not significantly different ($P<0.05$) between parts 1 and 2 of the experiment (Straw OMI: 27.9 *v.* 28.5 g/kg $LW^{0.75}$ per day, $s.e._{diff}$ = 2.6; total OMI: 42.0 *v.* 43.8 g/kg $LW^{0.75}$ per day, $s.e._{diff}$ = 3.6). Means from both parts were therefore used in subsequent analyses.

Total OMI increased linearly as the level of lucerne increased, whereas straw OMI was not affected (Tables 4.2 and 4.4A). The linear regression of total OMI

TABLE 4.2 Intake by rumen-fistulated sheep offered untreated barley straw supplemented with lucerne (Experiment 2).

Daily OMI g per kg $LW^{0.75}$	Diet description				
	US-0L	US-16L	US-32L	US-48L	s.e. means
Straw	31.5	26.9	27.8	26.9	2.88
Lucerne	0.0	5.6	13.8	25.9	-
Total	31.5	32.5	41.6	52.5	3.68

on lucerne OMI was highly significant ($P<0.01$) while that of straw OMI on lucerne OMI was not significant ($P<0.05$) The equations were:

Straw OMI = 29.3 - 0.09 (Lucerne OMI) r = 0.20 r.s.d. = 4.97
Total OMI = 29.3 + 0.91 (Lucerne OMI) r = 0.89 r.s.d. = 4.97

The soluble DM for the different batches of untreated straw and lucerne were similar; the respective means (±s.e.) of 87.4 (±4.0) and 379.8 (±3.8) g per kg were used in all *in sacco* studies. The soluble DM in ammonia-treated straw was 140.4 (±2.0) g per kg DM. Particle losses through the dacron bag pores were small for all feeds: 22.9 (±0.3); 16.1 (± 0.3) and 50.9 (± 4.5) g per kg DM for untreated straw, ammonia-treated straw and lucerne, respectively. In this and further experiments no correction for these losses was made because they were considered to be too small to affect the results.

The *in sacco* degradation of straw DM and lucerne DM in sheep offered diet US-32L was similar in Part 1 and Part 2 of the experiment (Table 4.3). A summary of the ANOVA to test the effect of the experimental part on *in sacco* degradation is given in Table 4.2A. For straw DM, there was a significant ($P<0.05$) part x incubation time interaction, but the difference between Part 1 and Part 2 was significant ($P<0.05$) only at 48 h. For diet US-32L, means from both parts of the experiment were therefore used in further analyses.

Mean values for the *in sacco* degradation of straw DM and lucerne DM are shown in Table 4.4. The corresponding ANOVAs are given in Table 4.3A. The *in sacco* degradation of straw DM and lucerne DM tended to increase as the level of lucerne increased, although the overall diet effect was non-significant. The *in sacco* degradation of straw DM at 11, 24 and 48 h was significantly higher ($P<0.01$) in period 2 than in period 1, with the values for 5 and 72 h being

TABLE 4.3 Effect of the experimental part on *in sacco* degradation of straw and lucerne DM (g/g) in sheep offered untreated barley straw supplemented with 320 g lucerne per kg dietary DM (Diet US-32L).

Feed	Part	Incubation times, h					
		5	11	24	48	72	Mean
Straw	1	0.103	0.189	0.341	0.455	0.546	0.327
	2	0.102	0.189	0.372	0.502	0.543	0.342
		NS	NS	NS	*	NS	NS
Lucerne	1	0.437	0.574	0.701	0.752	0.765	0.646
	2	0.449	0.614	0.702	0.743	0.764	0.654
		NS	*	NS	NS	NS	NS

s.e. of differences of means: between;
-parts across times and periods: straw = 0.0095; lucerne = 0.0063
-parts with the same time across periods straw: 0.0144;
lucerne = 0.0154 with corresponding 0.05 significant levels of t of 3.01 and 2.42.
NS: not significant; * $P<0.05$

TABLE 4.4 *In sacco* degradation (g/g) of straw DM and lucerne DM in sheep offered untreated barley straw supplemented with lucerne.

Feed/ Period	Incubation time, h	Diet description						
		US-0L	US-16L	US-32L	US-48L	100L	Mean	s.e. means
Straw/1	5	0.091	0.096	0.102	0.093	-	**0.095**	0.0055
	11	0.163	0.162	0.182	0.192	-	**0.175**	0.0090
	24	0.295	0.316	0.348	0.339	-	**0.325**	0.0310
	48	0.422	0.450	0.460	0.488	-	**0.455**	0.0142
	72	0.505	0.557	0.548	0.543	-	**0.538**	0.0087
	Mean	**0.295**	**0.316**	**0.328**	**0.331**	-	-	-
Straw/2	5	0.103	0.094	0.104	0.105	-	**0.102**	0.0040
	11	0.199	0.182	0.196	0.208	-	**0.196**	0.0083
	24	0.316	0.345	0.364	0.361	-	**0.347**	0.0174
	48	0.488	0.444	0.497	0.483	-	**0.478**	0.0269
	72	0.517	0.547	0.541	0.535	-	**0.535**	0.0122
	Mean	**0.324**	**0.323**	**0.341**	**0.338**	-	-	-
Lucerne/ 1 + 2	5	-	0.419	0.443	0.454	0.463	**0.445**	0.0080[1]
	11	-	0.536	0.594	0.582	0.554	**0.567**	0.0346
	24	-	0.664	0.702	0.713	0.691	**0.692**	0.0144
	48	-	0.747	0.747	0.749	0.731	**0.744**	0.0116
	72	-	0.764	0.761	0.761	0.752	**0.760**	0.0106
	Mean	-	**0.626**	**0.649**	**0.652**	**0.638**	-	-

s.e of differences of means between:
- -periods within the same time and diet: straw = 20.3; lucerne = 28.3
- -periods within the same time across diets: straw = 10.1; lucerne = 13.3
- -diets within the same time across periods: lucerne = 21.1
- -diets across times and periods : lucerne = 12.8
- -diets within the same time and period : straw = 23.3
- -diets within the same period across times : straw = 12.8

1. s.e. for diets US-16L, US-32L and US-48L only.

similar between incubation periods. The *in sacco* degradation of lucerne DM was similar between periods at all incubation times.

As the *in sacco* degradation of straw and lucerne was similar for all diets, the data were pooled and correlations between the degradation of DM and NDF in straw and between the degradation of DM and CP in lucerne calculated. Correlations for straw were initially calculated for each incubation period, but since both correlations were very similar the data were pooled. The correlations between the degradation of DM and NDF in straw and between the degradation of DM and CP in lucerne were both significant ($P<0.001$). Because of these high correlations it was considered unnecessary to present data on the *in sacco* degradation of straw NDF and lucerne CP in addition to the values for straw DM and lucerne DM given in Table 4.4. The following linear regressions can be used to predict the *in sacco* degradation of straw NDF and lucerne CP:

Straw NDF degr. = −0.080 + 1.1 (DM degr.) r = 0.996 r.s.d. = 0.015
Lucerne CP degr. = 0.017 + 1.2 (DM degr.) r = 0.998 r.s.d. = 0.023

The overall means for digestion kinetic parameters for straw and lucerne and the general pattern of the degradation-time curves for all data are shown in Figure 4.1. The effects of diet on digestion kinetic parameters for straw and lucerne were non-significant (See ANOVAs, Table 4.4A). Values for lag time and degradation rates were very variable among sheep (overall CV of 0.13 and 0.24 for straw and lucerne lag times and of 0.31 and 0.34 for the respective degradation rates). Potential degradability was less variable for lucerne (CV=0.04) than for straw (CV=0.12). For lucerne incubated in the rumen of sheep fed diet 100L, the lag time tended to be shorter (1.97 h), the potential degradability lower (0.752) and the degradation rate slower (0.079 per h) than

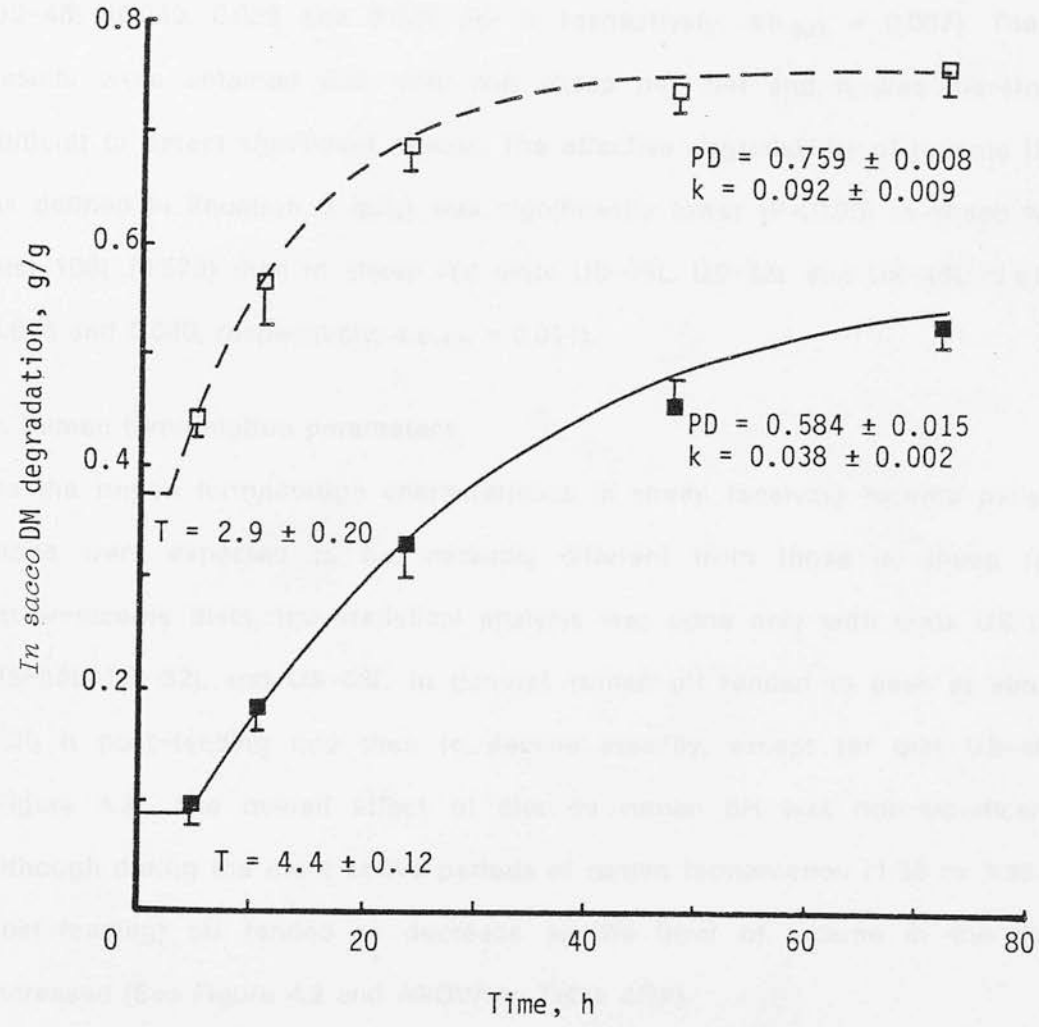

FIGURE 4.1 *In sacco* degradation curves and kinetic parameters for straw (■—■) and lucerne (□-□) incubated in the rumen of sheep offered untreated barley straw supplemented with increasing levels of lucerne.
T = lag time, h; PD = potential degradability, g/g; k = degradation rate, per h. ± indicates s.e. (Non-linear regressions calculated using 24 values for each incubation time; the symbols indicate means for each incubation time and the length of vertical bars the s.e. mean).

the overall means for diets US-16L, US-32L and US-48L (3.25 h, 0.761 and 0.097 per h).

b. Outflow rate of Cr-lucerne from the rumen

The fractional outflow rate of Cr-lucerne from the rumen was faster in sheep fed diet 100L (0.054 per h) than in sheep receiving diets US-16L, US-32L and US-48L (0.030, 0.039 and 0.028 per h, respectively; $s.e._{diff.}$ = 0.007). These results were obtained with only two sheep per diet and it was therefore difficult to detect significant effects. The effective degradability of lucerne DM as defined in Equation 6 (g/g) was significantly lower ($P<0.05$) in sheep fed diet 100L (0.575) than in sheep fed diets US-16L, US-32L and US-48L (0.615, 0.638 and 0.640, respectively; $s.e._{diff}$ = 0.011).

c. Rumen fermentation parameters

As the rumen fermentation characteristics in sheep receiving lucerne pellets alone were expected to be markedly different from those in sheep fed straw-lucerne diets, the statistical analysis was done only with diets US-0L, US-16L, US-32L and US-48L. In general, rumen pH tended to peak at about 1:30 h post-feeding and then to decline steadily, except for diet US-48L (Figure 4.2). The overall effect of diet on rumen pH was non-significant, although during the more active periods of rumen fermentation (1:30 to 3:30 h post-feeding) pH tended to decrease as the level of lucerne in the diet increased (See Figure 4.2 and ANOVA in Table 4.5A).

For all diets, NH_3-N increased from 0 to 1:30 h post-feeding and then decreased (Figure 4.2). Rates of decrease of NH_3-N after the peak were calculated as the slopes of the linear regressions of NH_3-N and time for each sheep. These rates were similar for all treatments, despite the higher peaks observed at 1:30 post-feeding for diets US-0L and US-48L. There was a

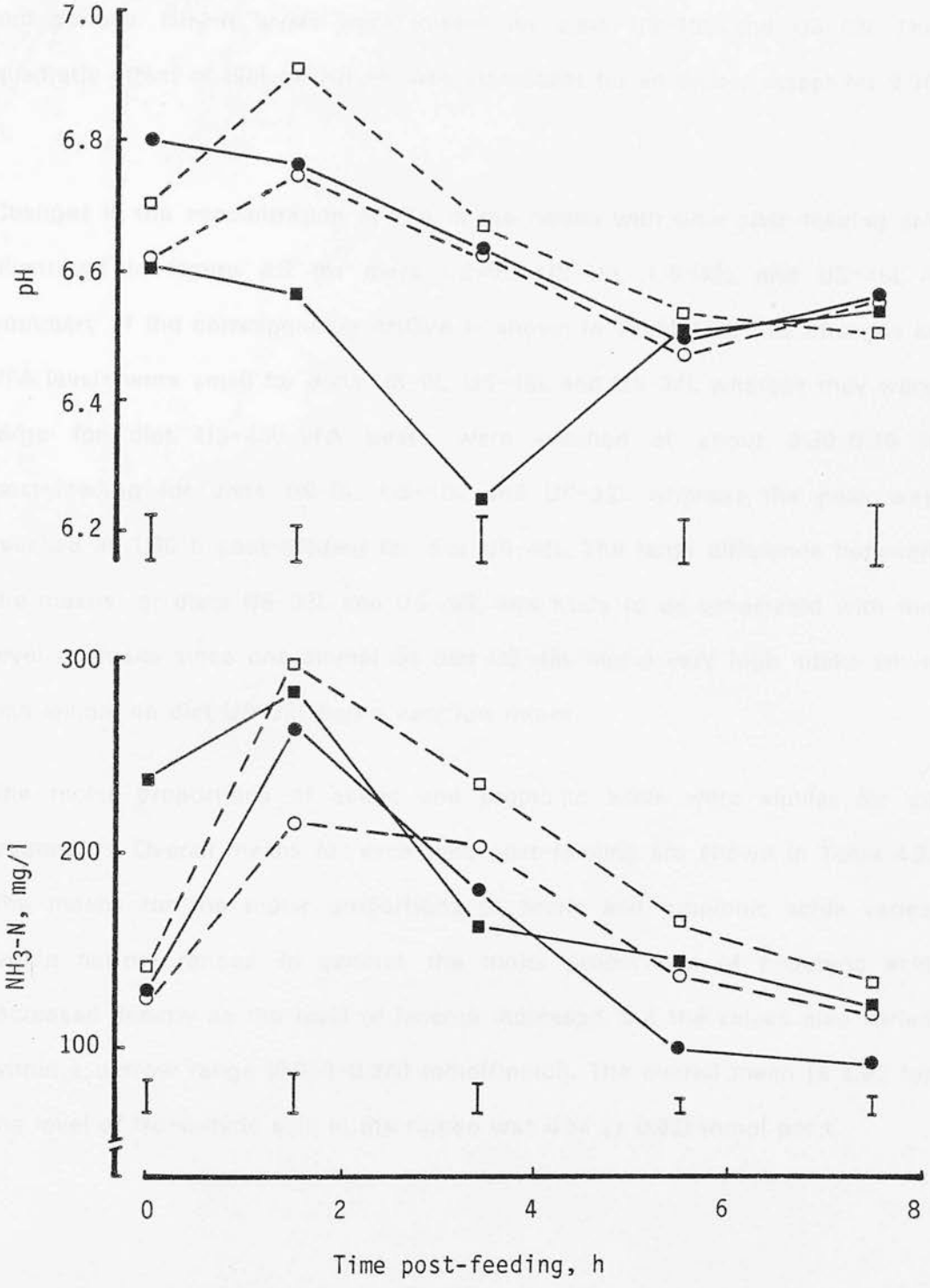

FIGURE 4.2 Changes in rumen pH and NH_3-N levels with time post-feeding in sheep offered untreated barley straw supplemented with lucerne. Diet US-0L (□--□); US-16L (○--○); US-32L (●-●) and US-48L (■-■). (The length of vertical bars indicates average s.e. of treatment means).

significant quadratic effect of diet on the mean NH_3-N for all sampling times and periods. NH_3-N levels were lowest for diets US-16L and US-32L. The quadratic effect of diet on NH_3-N was significant for all times, except for 3:30 h.

Changes in the concentration of VFA in the rumen with time post-feeding are illustrated in Figure 4.3 for diets US-0L, US-16L, US-32L and US-48L. A summary of the corresponding ANOVA is shown in Table 4.6A. The changes in VFA levels were small for diets US-0L, US-16L and US-32L whereas they were large for diet US-48L. VFA peaks were reached at about 3:30-5:30 h post-feeding for diets US-0L, US-16L and US-32L whereas the peak was reached at 1:30 h post-feeding for diet US-48L. The large difference between the means for diets US-32L and US-48L was likely to be associated with the level of intake since one animal on diet US-48L had a very high intake while one animal on diet US-32L had a very low intake.

The molar proportions of acetic and propionic acids were similar for all treatments. Overall means for each time post-feeding are shown in Table 4.5. The means for the molar proportions of acetic and propionic acids varied within narrow ranges. In general, the molar proportions of n-butyric acid increased linearly as the level of lucerne increased, but the values also varied within a narrow range (0.050-0.070 mmol/mmol). The overall mean (± s.e.) for the level of iso-butyric acid in the rumen was 0.54 (± 0.03) mmol per l.

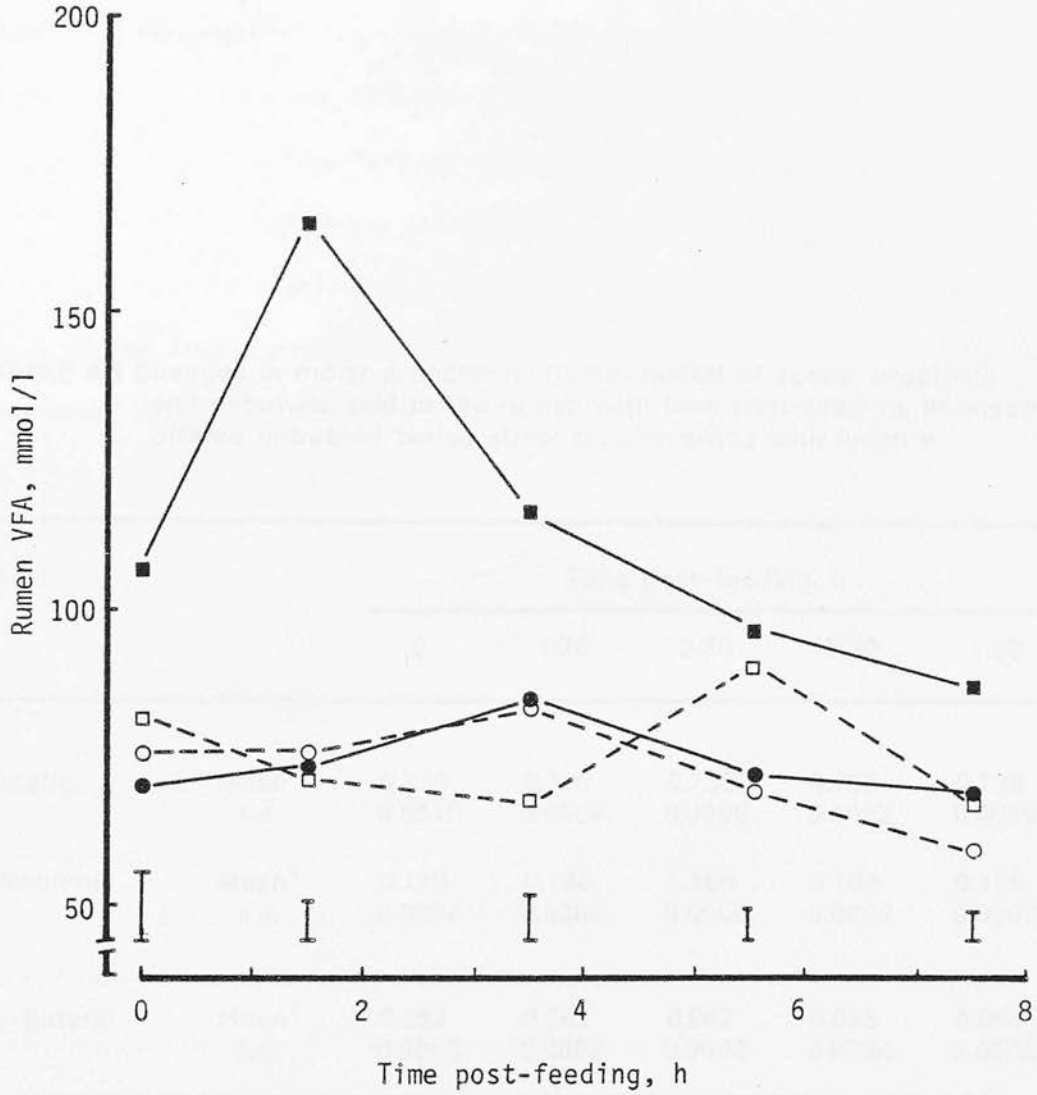

FIGURE 4.3 Relationship between rumen fluid VFA concentration and time post-feeding in sheep offered untreated barley straw supplemented with lucerne Diet US-0L (□-·-□); US-16L (○--○); US-32L (●—●) and US-48L (■—■).
(The size of vertical bars indicate average s.e. of treatment means).

TABLE 4.5 Changes in molar proportions (mmol/mmol) of acetic, propionic and n-butyric acid in the rumen with time post-feeding, in sheep offered untreated barley straw supplemented with lucerne.

Acid		Time post-feeding, h				
		0	1:30	3:30	5:30	7:30
Acetic	Mean[1]	0.729	0.729	0.736	0.735	0.736
	s.e.	0.0010	0.0007	0.0009	0.0013	0.0009
Propionic	Mean[1]	0.179	0.190	0.186	0.184	0.185
	s.e.	0.0007	0.0006	0.0006	0.0009	0.0007
n-Butyric	Mean[1]	0.064	0.061	0.062	0.065	0.064
	s.e.	0.0003	0.0002	0.0003	0.0004	0.0002

1. Overall mean for sheep an all dietary treatments.

4.3.1.2. Experiment 3: Intake and *in vivo* digestibility of diets containing untreated straw and lucerne

a. Intake

Total OMI and total DOMI increased linearly ($P<0.01$) with the level of lucerne in the diet whereas straw OMI was unaffected (Figure 4.4 and Table 4.7A). The linear regressions of total OMI on lucerne OMI and total DOMI on lucerne OMI were both highly significant ($P<0.01$) and explained about 0.6 of the total variation.The linear regression of straw OMI on lucerne OMI accounted for very little of the total variation. For every g increase in lucerne OMI, total OMI increased by about the same amount while total DOMI increased by about 0.6 g. The equations derived were:

Total OMI = 19.3 + 1.1 (Lucerne OMI) r = 0.74 r.s.d. = 6.9
Straw OMI = 19.3 + 0.1 (Lucerne OMI) r = 0.10 r.s.d. = 6.9
Total DOMI = 8.3 + 0.6 (Lucerne OMI) r = 0.76 r.s.d. = 3.9

where all intakes are expressed as g OM per kg $LW^{0.75}$ per day.

b. *In vivo* digestibility of the diets and N-balance

The chemical compositions of the feeds and the diets offered to the sheep are shown in Table 4.6. Total ash and CP in the diets increased whereas NDF and MADF decreased as the level of lucerne increased. The *in vivo* digestibilities of DM, OM and GE in the diets increased linearly ($P<0.05$) as the lucerne level increased (Figure 4.5; ANOVAs in Table 4.7A). On the other hand, NDF and MADF digestibility were similar for all treatments. There were no associative effects of straw and lucerne on total diet digestibility.

The ME of the diets and the ME intake increased linearly as the level of lucerne increased, the values were low for all treatments (Table 4.7; ANOVA in Table 4.7A). As the lucerne level in the diet increased, N intake, N excreted in

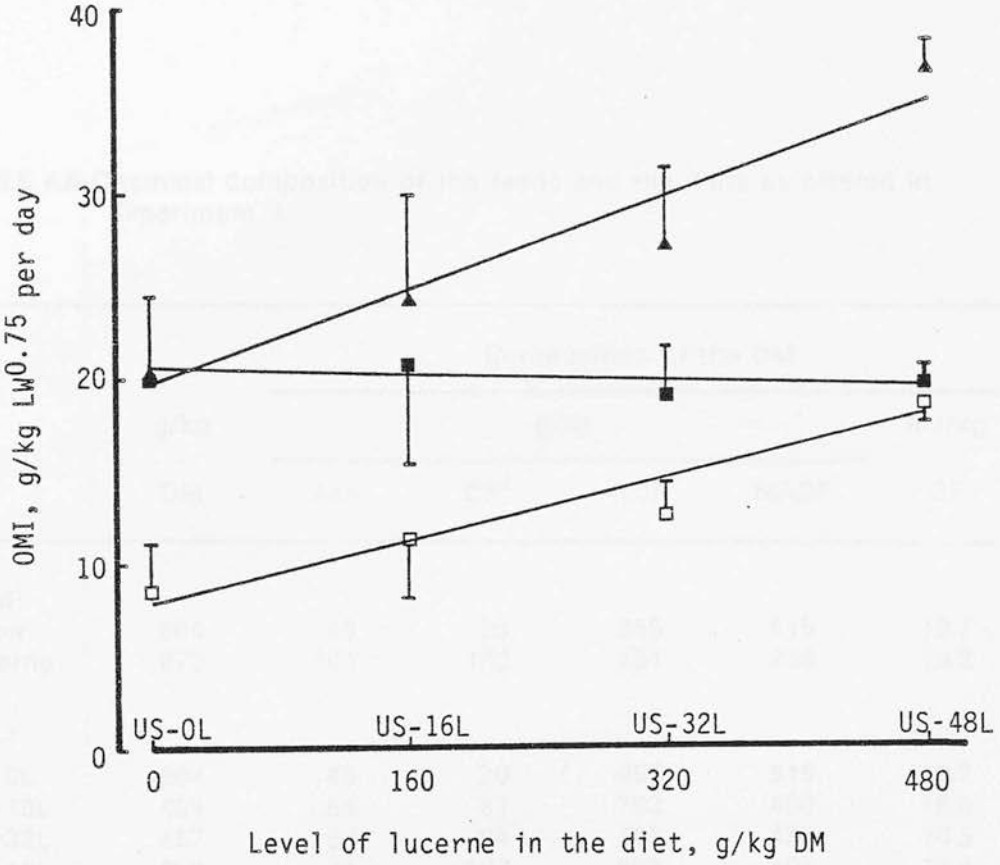

FIGURE 4.4 Relationships between the level of lucerne in the diet and: total OMI (▲—▲): straw OMI (■—■) and total DOMI (□—□) in sheep offered untreated barley straw and lucerne. (The linear effects were significant for total OMI and total DOMI only, see Table 4.7A; each symbol indicates the mean of 4 observations and the length of the vertical bars the s.e. mean).

TABLE 4.6 Chemical composition of the feeds and the diets as offered in Experiment 3.

	g/kg	Composition of the DM				MJ/kg
		g/kg				
	DM	Ash	CP[3]	NDF	MADF	GE
Feed[1]						
Straw	864	45	29	855	515	18.7
Lucerne	873	101	180	451	286	18.2
Diet[2]						
US-0L	864	45	70	855	515	18.7
US-16L	865	54	81	793	480	18.6
US-32L	867	64	94	702	436	18.5
US-48L	868	71	107	663	406	18.4

1. Determined composition.
2. Calculated values using the chemical composition of the feeds.
3. Including urea-N added to the diets.

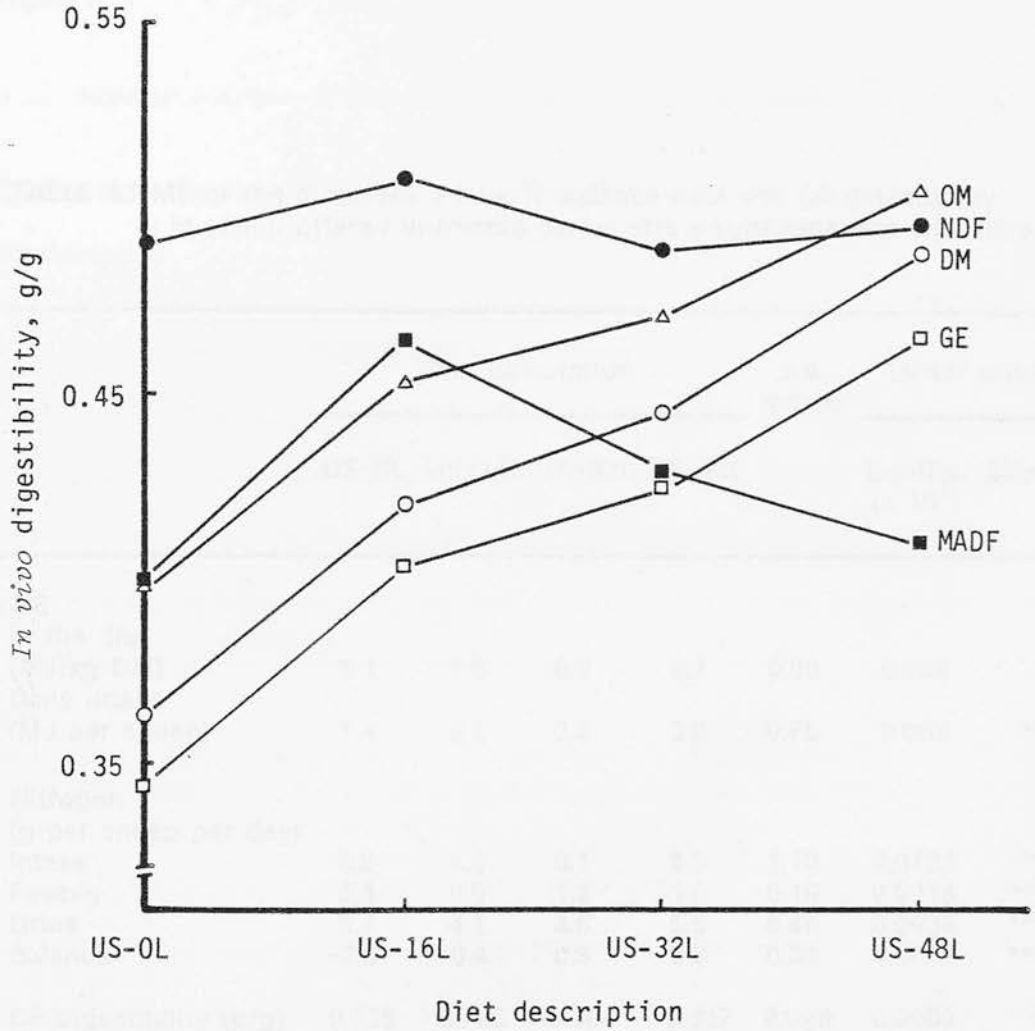

FIGURE 4.5 *In vivo* digestibility of the diets in sheep offered untreated barley straw supplemented with lucerne. (s.e. means: DM = 0.052; OM = 0.059; GE = 0.031; NDF = 0.051; MADF = 0.037).

TABLE 4.7 ME of the diets, ME intake, N-balance data and CP digestibility in sheep offered untreated barley straw supplemented with lucerne.

	Diet description				s.e. means	Linear effect	
	US-0L	US-16L	US-32L	US-48L		Coeffic. (x 10^5)	Signif.
ME							
In the diet (MJ/kg DM)	5.1	5.6	5.9	6.7	0.98	0.004	*
Daily intake (MJ per sheep)	1.4	2.1	2.4	3.6	0.75	0.004	**
Nitrogen (g per sheep per day)							
Intake	3.2	4.6	6.1	9.3	1.10	0.0122	***
Faeces	0.8	0.9	1.2	1.6	0.19	0.0016	***
Urine	3.7	4.1	4.6	5.5	0.40	0.0038	***
Balance	-1.3	-0.4	0.3	2.2	0.90	0.0067	***
CP digestibility (g/g)	0.718	0.790	0.804	0.827	0.026	0.0002	*
RDP/ME (g/MJ)[1]	12.1	10.9	12.0	12.5	-	-	-

1. Assuming rumen degradabilities of straw and lucerne CP of 0.50 and 0.82, respectively.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

faeces and urine, N balance and CP digestibility increased linearly. The sheep on diets US-0L and US-16L were in a negative N balance whereas the sheep on dietsUS-32L and US-48L were in a positive N balance. The RDP/ME ratios for all diets were above the requirements of the rumen microbes (ARC, 1984) (Table 4.7)

4.3.2. Supplementation of ammonia-treated straw with lucerne

4.3.2.1. Experiment 4: *In sacco* degradation of the feeds and rumen fermentation

a. Intake and *in sacco* degradation of the feeds

Total OMI increased linearly ($P<0.01$) as the level of lucerne in the diet increased, but straw OMI remained constant for all diets (Table 4.8, ANOVAs in Table 4.9A). The linear regression of straw OMI on lucerne OMI was non-significant ($P<0.05$) whereas that of total OMI on lucerne OMI was highly significant ($P<0.01$). The equations were:

Straw OMI = 33.9 + 0.01 (Lucerne OMI) r = 0.02 r.s.d. = 5.0
Total OMI = 33.9 + 1.00 (Lucerne OMI) r = 0.91 r.s.d. = 5.0

The *in sacco* degradation of straw was similar for all diets while the degradation of lucerne DM tended to increase as the level of lucerne in the diet increased (Table 4.9, ANOVAs in Table 4.8A). A highly significant ($P<0.01$) correlation was found between the *in sacco* degradation of DM and CP in lucerne when this was calculated with all data. It was therefore considered unnecessary to present results of lucerne CP degradation in addition to the results of lucerne DM degradation shown in Table 4.9. The following linear regression can be used to calculate lucerne CP degradation:

CP degr.= −0.090 + 1.29 DM degr. r = 0.992 r.s.d. = 0.020

TABLE 4.8 Intake by rumen-fistulated sheep offered ammonia-treated barley straw supplemented with lucerne (Experiment 4).

Daily OMI g per kg $LW^{0.75}$	Diet description				
	TS-0L	TS-16L	TS-32L	TS-48L	s.e. means
Straw	33.4	33.1	37.4	32.2	2.83
Lucerne	0.0	5.7	15.9	26.6	-
Total	33.4	38.8	53.3	58.8	2.90

TABLE 4.9 *In sacco* degradation (g/g) of straw DM and lucerne DM in sheep offered ammonia-treated barley straw supplemented with lucerne.

Feed/ Period	Incubation time, h	Diet description					
		TS-0L	TS-16L	TS-32L	TS-48L	Mean	s.e. means
Straw/ 1 + 2	5	0.159	0.157	0.164	0.165	**0.161**	0.0088
	11	0.276	0.278	0.268	0.293	**0.279**	0.0224
	24	0.472	0.423	0.463	0.447	**0.451**	0.0301
	48	0.629	0.578	0.580	0.632	**0.605**	0.0293
	72	0.696	0.615	0.682	0.696	**0.672**	0.0257
	Mean	**0.446**	**0.410**	**0.431**	**0.447**	-	-
Lucerne/ 1 + 2	5		0.432	0.449	0.460	**0.447**	0.0148
	11		0.567	0.572	0.638	**0.593**	0.0330
	24		0.662	0.705	0.701	**0.689**	0.0161
	48		0.736	0.751	0.746	**0.744**	0.0068
	72		0.751	0.752	0.761	**0.755**	0.0086
	Mean		**0.629**	**0.646**	**0.661**	-	-

s.e. of differences of means; between:
- -diets with the same time across periods: straw = 0.045; lucerne = 0.033
- -diets across times and periods: straw = 0.041; lucerne = 0.025

Digestion kinetic parameters for straw DM and lucerne DM calculated for each sheep and both incubation periods were similar among diets ($P<0.05$). Overall means for lag time (T), potential degradability (PD) and degradation rate (k) and degradation-time curves are shown in Figure 4.6 and the corresponding ANOVAs in Table 4.9A. T and k were quite variable among sheep (CV of 0.23 and 0.34 for straw DM and of 0.50 and 0.52 for lucerne DM, respectively). PD was more consistent among sheep (CV of 0.17 and 0.02 for straw DM and lucerne DM).

b. Outflow rate of Cr-lucerne from the rumen

The outflow rate of Cr-lucerne from the rumen tended to increase with the level of lucerne, though the differences were not significant ($P<0.05$). Means for diets TS-16L, TS-32L and TS-48L were 0.022, 0.032 and 0.034 per h ($s.e._{diff}$ = 0.010). Similarly, the effective degradability of lucerne DM did not differ ($P<0.05$) among diets, with means of 0.676, 0.670 and 0.674 for diets TS-16L, TS-32L and TS-48L, respectively ($s.e._{diff}$ = 0.013).

c. Rumen fermentation parameters

Diet and period effects were non-significant for pH and NH_3-N in the rumen (Figure 4.7, ANOVAs in Table 4.5A). Overall means for rumen pH varied within a narrow range. Rumen NH_3-N increased from 0 to 2 h post-feeding and then decreased for all diets. Rates of NH_3-N decrease after the peak, calculated as in Experiment 2, were similar for all treatments ($P<0.05$).

The total concentration of VFA in the rumen was not significantly affected by the diet ($P<0.05$) (Figure 4.8, ANOVA in Table 4.6A). The levels remained fairly constant up to 7:30 h post-feeding for diets TS-0L whereas they reached a peak at about 2-4 h for the other diets. In general, the molar proportions of acetic, propionic and n-butyric acid varied within narrow ranges. Overall

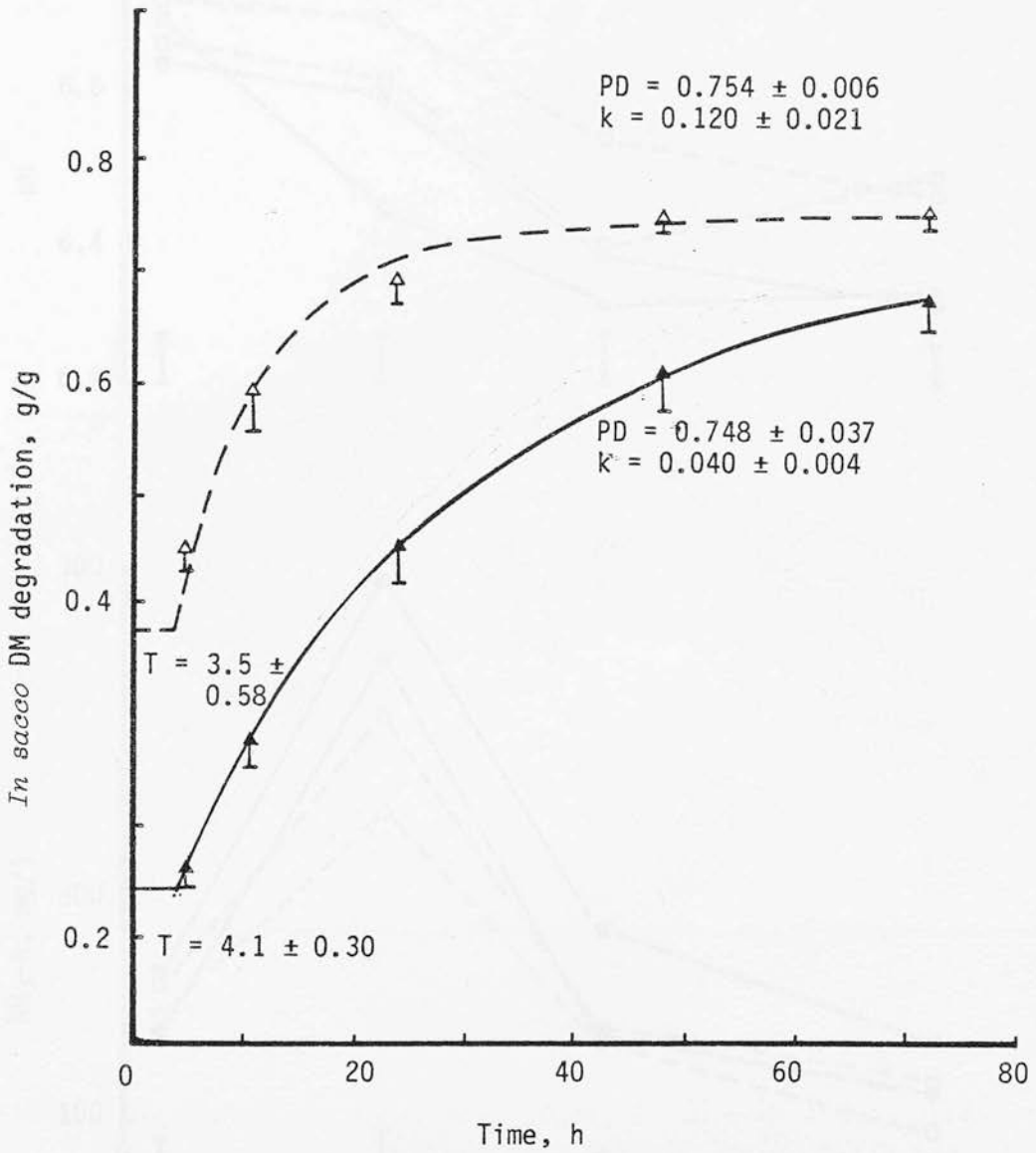

FIGURE 4.6 *In sacco* degradation curves and kinetic parameters for straw (▲—▲) and lucerne (△--△) incubated in the rumen of sheep offered ammonia-treated barley straw supplemented with increasing levels of lucerne.

T = lag time, h; PD = potential degradability, g/g
k = degradation rate, per h. ± indicates s.e.
(Non-linear regressions calculated using 24 values for each incubation time; the symbols indicate means for each incubation time and the length of vertical bars the s.e. mean).

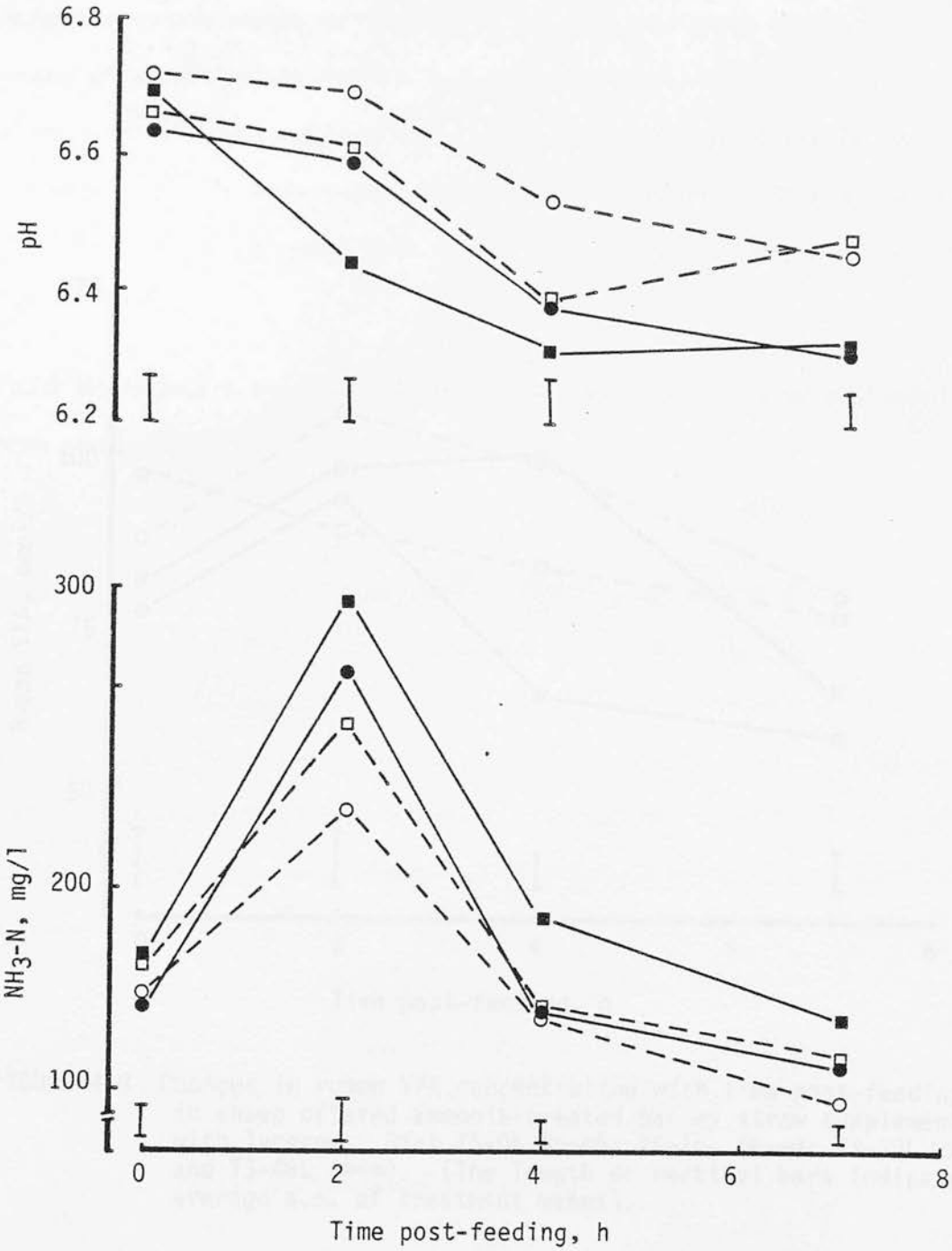

FIGURE 4.7 Changes in rumen pH and NH_3-N levels with time post-feeding in sheep offered ammonia-treated barley straw supplemented with lucerne. Diet TS-0L (□--□); TS-16L (●—●); TS-32L (○--○) and TS-48L (■—■). (The length of vertical bars indicates average s.e. of treatment means).

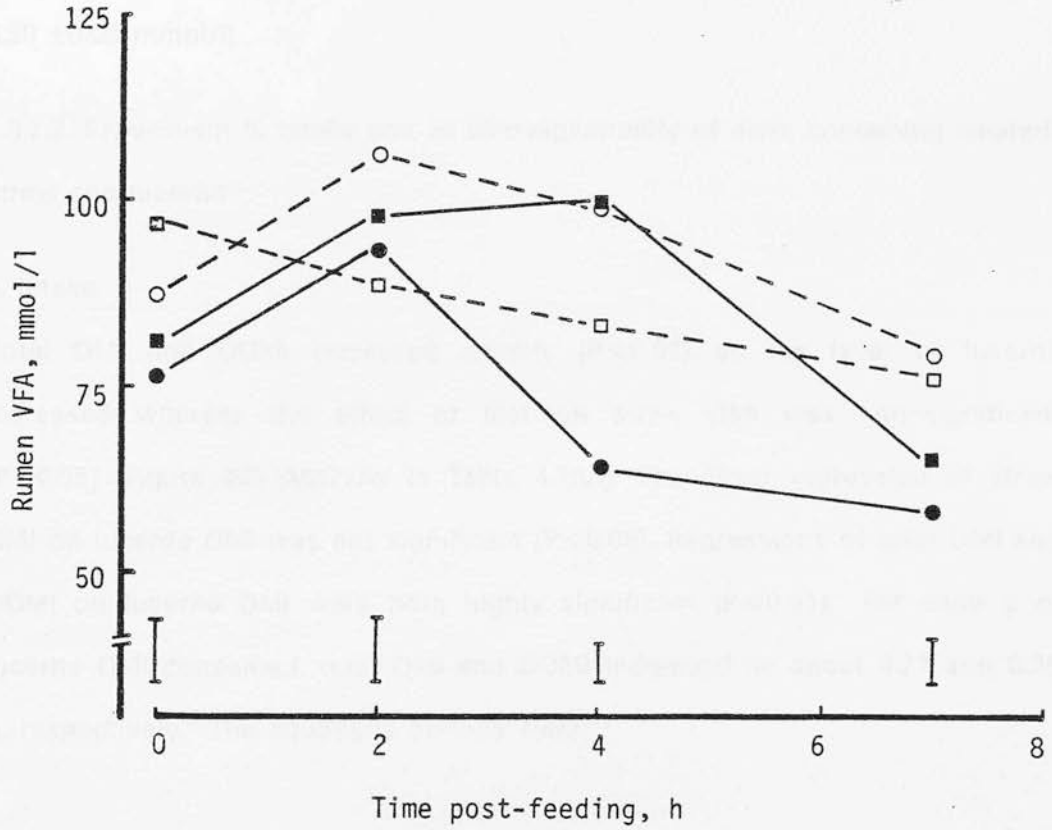

FIGURE 4.8 Changes in rumen VFA concentration with time post-feeding in sheep offered ammonia-treated barley straw supplemented with lucerne. Diet TS-0L (□--□); TS-16L (●–●); TS-32L (○–○) and TS-48L (■–■). (The length of vertical bars indicates average s.e. of treatment means).

means within post-feeding time are shown in Table 4.10 (ANOVAs are shown in Table 4.6A). The levels of the three acids were similar at all post-feeding times. The overall means for the molar proportions of acetic acid decreased linearly ($P<0.05$) whereas those of n-butyric acid increased linearly ($P<0.001$) as the level of lucerne in the diet increased. The overall means for molar proportions of propionic acid were similar for all diets. The levels of iso-butyric acid in the rumen fluid were low for all diets (Overal mean ±s.e = 0.39 ±0.03 mmol/l).

4.3.2.2. Experiment 5: Intake and *in vivo* digestibility of diets containing treated straw and lucerne

a. Intake

Total OMI and DOMI increased linearly ($P<0.01$) as the level of lucerne increased whereas the effect of diet on straw OMI was non-significant ($P<0.05$) (Figure 4.9, ANOVAs in Table 4.10A). The linear regression of straw OMI on lucerne OMI was not significant ($P<0.05$). Regressions of total OMI and DOMI on lucerne OMI were both highly significant ($P<0.01$). For each g of lucerne OMI consumed, total OMI and DOMI increased by about 0.77 and 0.35 g, respectively. The equations derived were:

Total OMI = 35.7 + 0.77 (Lucerne OMI) r = 0.80 r.s.d. = 5.9
Straw OMI = 35.7 - 0.23 (Lucerne OMI) r = 0.36 r.s.d. = 5.9
Total DOMI = 21.5 + 0.35 (Lucerne OMI) r = 0.69 r.s.d. = 3.8

where all intakes are expressed as g OM per kg $LW^{0.75}$ per day

TABLE 4.10 Changes in molar proportions (mmol/mmol) of acetic, propionic and n-butyric acid in the rumen with time post-feeding, in sheep offered ammonia-treated barley straw supplemented with lucerne.

Acid		Time post-feeding, h			
		0	2	4	7
Acetic	Mean[1]	0.729	0.737	0.741	0.716
	s.e.	0.0008	0.0009	0.0008	0.0011
Propionic	Mean[1]	0.189	0.185	0.187	0.202
	s.e.	0.0004	0.0006	0.0007	0.0006
n-Butyric	Mean[1]	0.061	0.061	0.058	0.067
	s.e.	0.0003	0.0002	0.0002	0.0004

1. Overall mean for sheep on all dietary treatments.

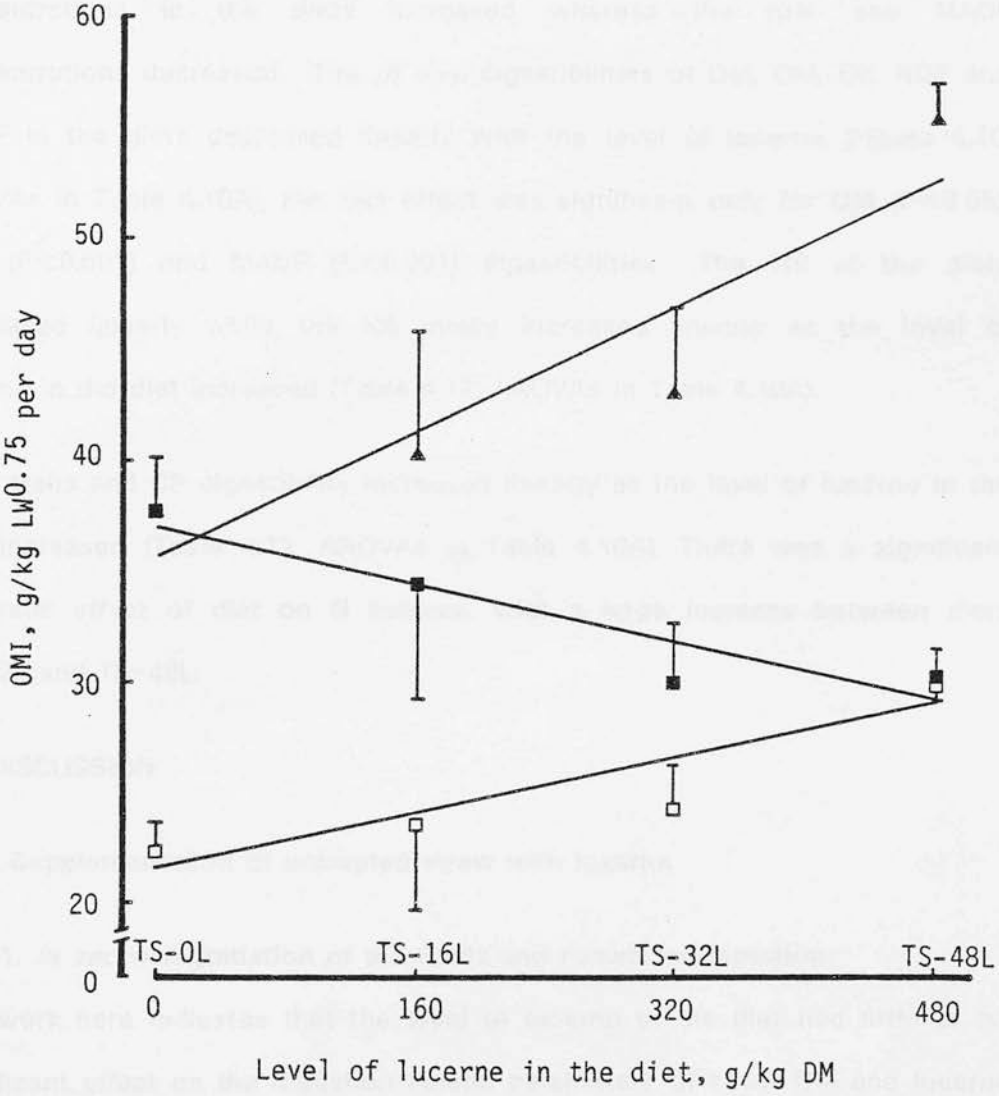

FIGURE 4.9 Relationships between the level of lucerne in the diet and: total OMI (▲—▲); straw OMI (■—■) and total DOMI (□—□) in sheep offered ammonia-treated barley straw and lucerne. (The linear effects were significant for total OMI and total DOMI only, see Table 4.10A, each symbol indicates the mean of 4 observations and the length of vertical bars the s.e. mean).

b. *In vivo* digestibility of the diets and N-balance

The chemical composition of the feeds and the diets offered to the sheep is shown in Table 4.11. As the level of lucerne increased, the ash and CP concentrations in the diets increased whereas the NDF and MADF concentrations decreased. The *in vivo* digestibilities of DM, OM, GE, NDF and MADF in the diets decreased linearly with the level of lucerne (Figure 4.10, ANOVAs in Table 4.10A), but this effect was significant only for OM ($P<0.05$), NDF ($P<0.001$) and MADF ($P<0.001$) digestibilities. The ME of the diets decreased linearly while the ME intake increased linearly as the level of lucerne in the diet increased (Table 4.12, ANOVAs in Table 4.10A).

All N traits and CP digestibility increased linearly as the level of lucerne in the diet increased (Table 4.12, ANOVAs in Table 4.10A). There was a significant quadratic effect of diet on N balance, with a large increase between diets TS-32L and TS-48L.

4.4. DISCUSSION

4.4.1. Supplementation of untreated straw with lucerne

4.4.1.1. *In sacco* degradation of the feeds and rumen fermentation

The work here indicates that the level of lucerne in the diet had little or no significant effect on the digestion kinetic parameters of straw DM and lucerne DM. However, the CV for these parameters were very high. The number of sheep per treatment would have had to be doubled for differences of 0.2 of the means of lag time (T) and potential degradability (PD) to become statistically significant ($P<0.05$). To detect a significant difference of 0.2 in degradation rate (k), the number of sheep per treatment would have had to be increased twelve fold. One bag for each of five incubation times, in three

TABLE 4.11 Chemical composition of the feeds and the diets as offered in Experiment 5.

	g/kg	Composition of the DM				
		g/kg				MJ/kg
	DM	Ash	CP[3]	NDF	MADF	GE
Feed[1]						
Treated Straw	950	50	96	782	523	18.8
Lucerne	898	102	161	455	297	18.3
Diet[2]						
TS-0L	950	50	96	782	523	18.8
TS-16L	942	58	105	732	488	18.7
TS-32L	934	66	116	679	452	18.6
TS-48L	926	74	126	629	417	18.5

1. Determined composition.
2. Calculated values using the chemical composition of the feeds.
3. Including urea-N added to the diets.

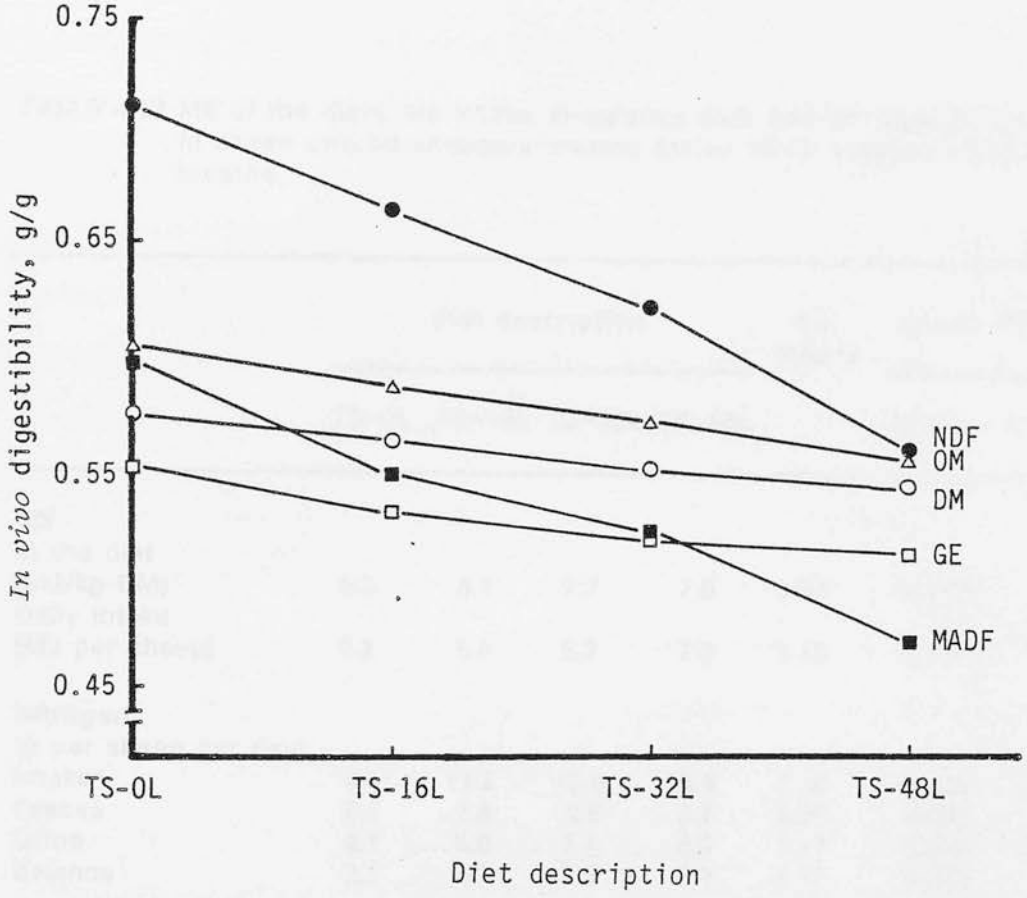

FIGURE 4.10 *In vivo* digestibility of the diets in sheep offered ammonia-treated barley straw supplemented with lucerne. (s.e. means: DM = 0.028; OM = 0.028; GE = 0.015; NDF = 0.027; MADF = 0.017).

TABLE 4.12 ME of the diets, ME intake, N-balance data and CP-digestibility in sheep offered ammonia-treated barley straw supplemented with lucerne.

	Diet description				s.e. means	Linear effect	
	TS-0L	TS-16L	TS-32L	TS-48L		Coeffic.	Signif.
ME							
In the diet (MJ/kg DM)	8.5	8.1	7.7	7.6	0.51	-0.002	*
Daily intake (MJ per sheep)	5.2	5.5	5.7	7.1	1.15	0.004	*
Nitrogen (g per sheep per day)							
Intake	9.4	11.2	13.8	18.9	2.10	0.020	***
Faeces	2.1	2.5	2.8	3.2	0.39	0.002	**
Urine	4.1	5.0	7.4	8.6	1.17	0.010	***
Balance[1]	3.2	3.7	3.6	7.1	1.17	0.008	**
CP digestibility (g/g)	0.771	0.771	0.791	0.832	0.014	$1.3X10^{-4}$	*
RDP/ME (g/MJ)[2]	7.9	9.7	11.5	13.1	-	-	-

1. The deviations from linearity were significant (See Table 4.10A).
2. Assuming rumen degradabilities of 0.50 and 0.82 for the CP in the original straw and lucerne, respectively and of 0.70 for the CP added through ammoniation.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

sheep, for two periods may be sufficient to obtain acceptable variation for the *in sacco* degradation of feeds (Mehrez and Ørskov, 1977). However, this does not necessarily apply when digestion kinetic parameters are estimated by non-linear regression analysis of data for *in sacco* degradation. Generally PD was less variable than T and k. As samples of the same feeds were incubated in the rumen of all sheep, this reduced variation may indicate that PD is an attribute of the feed itself (Mertens and Ely, 1982). On the other hand, T and k may be more closely related to the microbial activity in the rumen of each animal. Different forages differ in their lag time when incubated in the same microbial environment (Mertens and Ely, 1982). The lag time of the same forage can be altered by changing the degrading capacity of cellulolytic bacteria (Mertens and Loften, 1980; Miller and Muntifering, 1985). It therefore seems that both substrate- and microbial-related factors influence lag time.

The very high correlations obtained between the *in sacco* degradation of DM and NDF in straw and DM and CP in lucerne are of interest. Many resources and much effort could be saved by using DM degradation to predict NDF or CP degradation. In a variety of feeds, including lucerne, Varga and Hoover (1983) also reported high correlations between the *in sacco* degradation of NDF and DM. Similarly, Ahmed (1982) found a correlation of r=0.99 between the *in sacco* disappearence of DM and N in soya bean and fish meals. Nocek *et al* (1979) reported a positive correlation (r=0.78) between the rates of degradation for CP and DM in a wide variety of feeds, including dried lucerne.

The values for the effective rumen degradability of lucerne DM (dg, Equation 6, Section 4.2.8) were within the range of values for the *in sacco* degradation of lucerne DM at incubation times of 5, 11, 24, 48 and 72 h (Table 4.4). The *in sacco* degradation of lucerne CP at any time can be predicted with good

accuracy from the *in sacco* degradation of lucerne DM since these variables were highly correlated. Therefore, the dg of lucerne CP can be predicted with reasonable accuracy from its DM dg. For example, the mean dg of lucerne CP in sheep offered diets US-16L, US-32L and US-48L was 0.771. The dg of lucerne CP in the rumen of sheep fed lucerne pellets alone (Diet 100L) was 0.729. These values are very similar to values reported elsewhere for the degradability of lucerne CP in the rumen of sheep fed lucerne only, determined *in vivo* (0.72-0.80; Lindsay and Hogan, 1972; Mathers and Miller, 1981; Nolan and Leng, 1972 ; Pilgrim *et al*, 1970) and *in sacco* (0.71-0.73; Mathers and Miller, 1981; Miller, 1980) The dg of lucerne CP was higher for diets US-16L, US-32L and US-48L than for diet 100L. This was a result of the slower degradation rate and faster outflow rate of lucerne from the rumen of sheep fed diet 100L. The fibre-degrading bacteria in the rumen of sheep fed straw-lucerne diets may have being more active than in the rumen of sheep fed diet 100L, being capable of attacking more refractory protein fractions (Ganev *et al*, 1979).

Rumen pH varied within a narrow range for all diets. There was a tendency for pH to decrease with increasing levels of lucerne in the diets at the times of more active rumen fermentation. This is likely to be due to the release of the more easily fermentable fractions in the lucerne. Similar results were reported by Soofy *et al* (1983) for lucerne-soya bean stover diets. The NH_3-N concentrations in the rumen were almost invariably above 100 mg per l, indicating an adequate supply to the rumen microbes (Buttery, 1977). The significant quadratic effects of the lucerne level on rumen NH_3-N at most post-feeding times are difficult to explain. The decrease in rumen NH_3-N from diet US-0L to diets US-16L and US-32L may be associated with the rate of release of RDP from lucerne and urea. Although the total dietary concentration

of RDP increased from diet US-0L to diet US-32L, the proportion of RDP supplied by urea decreased whereas that supplied by lucerne increased. It is likely that the lucerne RDP was released in the rumen at a slower rate than the urea RDP. This slower release of lucerne RDP may have also been more commensurate with the release of fermentable energy from lucerne and/or the straw, promoting a faster growth of the rumen bacteria and therefore a higher uptake of NH_3 from the rumen fluid. The increase in rumen NH_3-N between diets US-32L and US-48L may indicate a surplus of NH_3-N above the microbial requirements.

4.4.1.2. Intake and *in vivo* digestibility of the diets

In experiments 2 and 3, feeding lucerne did no result in a reduction in straw intake, *i.e.* the lucerne was acting as a true supplement. These results differ from results reported in the literature (See Table 2.8 and Figures 2.10 and 2.11); where the average replacement rate (decrease in g of OMI of low-quality roughage for each g of high-quality roughage OMI) was about 0.7 for levels of total intake similar to those observed in the present trials. The increase of 0.6 g DOMI per g lucerne OMI observed in Experiment 3 (Figure 4.4) was much higher than that for DDMI calculated from the literature data (0.32 g DDMI per g high-quality roughage DMI, Figure 2.11). This difference could be due to a synergistic effect of feeding the lucerne in a ground and pelleted form and adjusting the RDP of the diets to supply the requirements of the rumen microbes. In contrast, in the experiments reviewed (Table 2.8) the supplementary high-quality roughages were fed long or coarsely chopped and the RDP of the diets was not adjusted.

The importance of the effects of the physical capacity of the rumen and the microbial activity on the digestion of roughages was clearly emphasized in the

literature review (Sections 2.4 and 2.5.2.1). Density and fibre concentration in forages are associated with limitations of their intake by ruminants (Van Soest, 1982). The maximum NDF intake in sheep consuming forages is about 35 g per kg $LW^{0.75}$ per day (Figure 17.13 of Van Soest, 1982). The lack of replacement of straw by lucerne in Experiment 3 could have been due not only to an effect of feeding the lucerne ground and pelleted but also to the fact that the NDF intakes by the sheep on all diets (18.2 to 26.3 g NDF per kg $LW^{0.75}$ per day from diet US-0L to diet US-48L) were below this maximum. No apparent reasons could be found for such low levels of intake. Supplementation of straws with lucerne pellets in sheep having intakes of straw NDF closer to the maximum of 35 g per kg $LW^{0.75}$ may result in some replacement of straw by lucerne. The activity of the fibre-degrading microbes in the rumen was expected to be close to optimum under the conditions of the present experiments for the following reasons: a) the RDP/ME ratios in the diets were adequate; b) low levels of iso-butyric acid were detected in the rumen of all sheep (Experiment 2); c) preformed aminoacids were likely provided by lucerne CP and/or microbial protein and; d) rumen pH was always above the crucial threshold below which cellulolysis is inhibited (Mould *et al*, 1983).

The *in vivo* digestibility and the ME of the straw used in Experiment 3 was very low compared to values reported in the literature for barley straw (Table 2.5). These results may be related to the chemical composition of the straw which had concentrations of NDF and MADF higher than the mean values reported in the literature (Table 2.4).

Assuming a constant lucerne OMD of 0.611 (Table 6.11, Chapter 6), it can be calculated that the straw OMD was similar at all levels of lucerne supplementation (0.399, 0.426, 0.397 and 0.415 for diets US-0L, US-16L, US-32L

and US-48L). As indicated previously, lucerne was acting as a supplement. The similar digestibilities of NDF and MADF for all diets can be explained by the fact that the digestibilitiesof these fractions in straw and lucerne were similar (0.491 and 0.487 for NDF and 0.400 and 0.386 for MADF in straw and lucerne, respectively, see Figure 4.5 and Table 6.11).

4.4.1.3. Crude protein digestibility and nitrogen balance

The high *in vivo* digestibility of the CP in the diets was expected since most CP was supplied by urea and lucerne. Urea is completely degraded in the rumen and the digestibility of lucerne CP was expected to be about 0.8 (calculated value from data in Table 6.11, Chapter 6), which is within the range of values reported elsewhere (0.69-0.83 ; Coelho da Silva, Seely, Thomson, Beever and Amstrong, 1972; Egan *et al*, 1975; Mathers and Miller, 1981; Thomson and Cammel, 1979).

The addition of increasing levels of lucerne to the straw was associated with a linear increase in N balance. Positive N balances were almost achieved when the CP concentration of the diet was about 80 g per kg DM (Diet US-16L). This is within the range of values from the work of Milne and Bagley (1976), Mosi and Butterworth (1985) and Siebert and Kennedy (1972). In the experiments of these authors, positive N balances were observed in sheep consuming diets based on low-quality roughages and legume and grass forages with CP concentrations between 42 and 95 g per kg DM. This wide variation is probably associated with effects of N recycling into the rumen and differences in levels of intake of digestible nutrients on the efficiency of absorption and utilization of dietary CP (Oldham, 1984) and the growth of bacteria in the rumen which influences the amount of bacterial CP digested and absorbed in the hindgut.

4.4.2. Supplementation of ammonia-treated straw

4.4.2.1. *In sacco* degradation of the feeds and rumen fermentation

The lack of effect of the level of lucerne in the diet on the *in sacco* degradation of treated straw may be associated with the microbial activity in the rumen. The fibre-degrading capacity of the rumen microbes in sheep fed treated straw alone would have been expected to be high (Silva and Ørskov, 1984), with little scope for further improvement.

The digestion kinetic parameters for treated straw and lucerne were not significantly affected by the level of lucerne in the diet. However, as for untreated straw (Experiment 3), lag time and degradation rate varied considerably among sheep. On the other hand, the potential degradability (PD) for treated straw and lucerne was similar among sheep and this supports the previous suggestion that this parameter is an attribute of the feed itself.

As with sheep fed untreated straw-based diets a highly significant correlation between the *in sacco* degradation of DM and CP in lucerne was found with sheep receiving diets based on ammonia-treated straw (Experiment 4). This expands the range of diets and levels of intake where predictive equations could be used to calculate CP degradabilities from the more easily obtainable DM data. The effective degradability of the lucerne CP (dg), as estimated in Experiment 2, had an overall mean of 0.778. This is very similar to the value obtained in Experiment 2 (0.771).

Rumen parameters followed trends similar to those described for untreated straw-based diets. The level of lucerne in the diet had a similar quadratic effect on the rumen NH_3-N, although this effect was not significant. Despite an increasing supply of total RDP, rumen NH_3-N tended to decrease as the

lucerne level increased to 160–320 g per kg of dietary DM (Diets TS–16 and TS–32L). This again may reflect a progressively improved synchronisation between NH_3–N release and uptake by a faster growing bacterial population. Above 160–320 g lucerne per kg of dietary DM, microbial growth may have reached a limit with a resulting accumulation of NH_3 in the rumen.

4.4.2.2. Intake and *in vivo* digestibility of the diets

The intake results from the *in sacco* degradation study with 60–kg sheep are similar to those for untreated straw (Experiment 2). There was no replacement of treated straw by lucerne and this compares very favourably with the results shown in Figure 2.10. Results for chemically–upgraded roughages were not included in Figure 2.10, but total DM intakes were similar to those in this trial. However, in Experiment 5 with smaller sheep (40 kg) the OMI of treated straw tended to decrease as the level of lucerne increased. The increase of 0.35 g of total DOMI for each g of lucerne OMI was higher than the respective values in Figure 2.10. The apparent replacement effects found here could be related to the fact that the total NDF intake by sheep consuming treated straw alone was near the maximum NDF intake calculated from data presented by Van Soest (1982) for sheep consuming a wide variety of forages (35 g NDF per kg $LW^{0.75}$). Therefore, the increasing NDF intake resulting from increasing the level of lucerne in the diet could have only been accommodated in the rumen by reducing the straw intake and/or increasing the rate of passage of digesta. In Experiment 5, the *in vivo* digestibility of NDF in the diets decreased linearly with the level of lucerne supplementation. This was likely to be due to changes in the rate of passage of digesta and not to changes in the rumen degradation of NDF. The *in sacco* degradations of straw and lucerne were not affected by the level of lucerne in the diet (Experiment 4). Moreover, it is likely that the increased total intake resulting from increasing the level of lucerne in

the diet was associated with increasing rates of passage of digesta through the gastro-intestinal tract (Grovum and Williams, 1977).

The *in vivo* digestibilities of OM and DM in the treated straw and the increase in these variables due to ammoniation were similar to results reported in the literature for barley straw fed to sheep (Dryden and Kempton, 1983; Sundstøl, 1984; Sundstøl, Coxworth and Mowatt, 1978; Williams, 1984). To assess the effects of ammoniation, the results for the *in vivo* digestibilities of OM and DM in treated straw (0.603 and 0.574, respectively, Figure 4.10) were compared with results for the corresponding untreated straw (Batch 2, Table 4.1) determined by Ali (1985) (0.512 and 0.498 for OMD and DMD, respectively). The significant linear decrease in the digestibilities of OM, NDF and MADF in the diets resulting from increasing the level of lucerne (Figure 4.10) suggests the existence of a negative associative effect of straw and lucerne. As indicated previously, this effect was likely to be due to the effects of the lucerne pellets on increasing the rate of passage of digesta. As was expected the negative associative effects were more marked for the fractions which are degraded at slow rates; *i.e.* NDF and MADF (Van Soest, 1982). The present results are similar to those of Al-Ani, Zorrilla-Rios and Horn (1984) who found that as the level of supplementation with lucerne hay or dried lucerne meal increased, the *in vivo* digestibility of fibre in the diet decreased linearly when ammoniated wheat straw was the basal roughage, but remained constant when untreated straw was the basal roughage.

4.4.2.3. Crude protein digestibility and nitrogen balance

As might be expected the digestibility of CP in the diets based on treated straw and lucerne (Experiment 5) was high. As indicated previously, the digestibility of CP in lucerne would have been expected to be about 0.8 and

about 0.85 of the CP added to the straw through ammoniation was likely to be degraded in the rumen (Abidin and Kempton, 1981; Dryden and Kempton, 1983). The large difference for N-balance between diets TS-32L and TS-48L (Table 4.12) is largely unexplained. A steady linear increase in N-balance would have been expected as a result of increasing the level of lucerne since this was associated with increasing intakes of N and digestible OM (Oldham, Buttery, Swan and Lewis, 1977). However, as pointed in the literature review, N-retention data should be interpreted with caution. These data are usually overestimated due to inevitable losses of N during storage and handling of faeces and urine and losses of N in wool and scurf which are not taken into account in conventional balance trials.

4.4.3. General

The intake of the straws by the sheep in all experiments was low (9-14 g DM per kg LW per day). Even at the highest level of lucerne supplementation of untreated straw, the small 33-kg sheep (Experiment 3) were not able to obtain their estimated ME requirements for maintenance. Using the ME of the diets from the *in vivo* trial (Experiment 3), it can be calculated that the 60-kg sheep could obtain their maintenance requirements for ME from all diets, except from the straw alone. The ME requirement for maintenance of the 40-kg sheep fed treated straw (Experiment 5) was satisfied only with diet TS-32L. A surplus of about 0.25 over this requirement was observed with diet TS-48L. Larger sheep in the respective *in sacco* study (Experiment 4) were able to get from 1.2 to 1.5 of their ME requirements for maintenance with all diets. Although these results are from different experiments, they suggest that straw-lucerne diets could be more suitable for older and heavier animals. Cattle, with higher intakes of digestible nutrients relative to body size (See Section 2.6.1.2) should be able to obtain more digestible nutrients from diets based on straw and

lucerne. Indeed, Playne (1970) observed that a low-quality grass provided an above-maintenance diet for cattle but a below-maintenance diet for sheep.

4.5. CONCLUSIONS

a. Lucerne pellets can be included up to 0.48 in diets based on untreated and ammonia-treated barley straw fed to sheep with the following effects: significant increases in the intake of digestible nutrients and small or no effects on the *in sacco* degradation of the dietary components, rumen fermentation parameters and straw intake.

b. The effects of lucerne supplementation on the *in vivo* digestibility of the dietary components vary according to the feeding value of the straw. Increasing the level of lucerne pellets results in no apparent change in the digestibility of untreated straw whereas it results in reductions in the digestibility of treated straw.

c. Lucerne supplementation in diets based on barley straws fed to sheep seems to have no effect on the outflow of undigested lucerne particles from the rumen and the effective degradability of lucerne CP. Lucerne is a good source of RDP with a high degradability of CP in the rumen (0.77 for the type of diets used in this work).

d. The significant increase in total intake resulting from increasing the level of lucerne supplementation helps overcome the major constraint in the utilisation of cereal straws by ruminants, *i.e.* the low intake of digestible nutrients.

CHAPTER 5

PARTICLE SIZE REDUCTION AND RETENTION OF STRAW IN THE RETICULO-RUMEN OF SHEEP OFFERED STRAW-LUCERNE DIETS

5.1. INTRODUCTION

The experiments described in the preceeding chapter were focussed on the intake and digestibility of some dietary components in sheep offered barley straws supplemented with lucerne. In one experiment with ammonia-treated straw, the *in vivo* digestibility of the total diet decreased with increasing levels of lucerne supplementation and this may have been associated with a shorter retention time of digesta in the alimentary tract. With this type of diet, the rate of passage of digesta from the reticulo-rumen is important in controlling roughage intake and digestibility.

It is a generally accepted hypothesis that the control of intake of long roughage is related mainly to the rate of size reduction of feed particles in the reticulo-rumen and their rate of passage from this organ (Balch and Campling, 1962; Campling, 1970; Welch, 1982). However, this hypothesis has not been tested extensively. This is mostly due to the difficulty of measuring rates of size reduction of large feed particles in the rumen (Chesson and Ørskov, 1984). This subject and the development of techniques for particle size analysis of feed, digesta and faeces have been clearly identified as being in need of more extensive research (Baldwin *et al*, 1977; Mertens and Ely, 1979; Weston and Kennedy, 1984).

The objective of this work was to investigate the effect of ammonia treatment and lucerne supplementation of barley straw in diets for sheep on intake and physical breakdown of chromium-mordanted straw particles in the reticulo-rumen and their passage to the hindgut.

5.2. MATERIALS AND METHODS (Experiment 6)

5.2.1. Animals and management

Twelve Suffolk-cross wethers (58.4 ±1.4 kg LW) fitted with permanent rumen cannulae (40 mm diameter) were used. They were harnessed for collection of faeces and urine and kept in individual crates under continuous illumination.

5.2.2. Diets and feeding

One of the following diets was fed *ad libitum* to each group of three sheep: untreated straw alone (US); untreated straw + 480 g lucerne pellets DM per kg of total dietary DM (US-480); ammonia-treated straw alone (TS) and ammonia-treated straw + 480 g lucerne pellets DM per kg of total dietary DM (TS-480). The straws (*c.v.* Golden Promise) were coarsely shredded in a small-tub grinder. Treatment of the straw was done in an oven for 23 h with a peak temperature of 90 °C. Anhydrous ammonia at about 35 g NH_3 per kg straw DM (Sundstøl, Kossila, Theander and Thomsen, 1978) was used.

The daily allowance of feed was offered in equal amounts at 9:00 and 17:00 h. For diets US-480 and TS-480 the straw was offered after the pellets were completely consumed. In all treatments,the amount of straw offered was about 0.35 in excess of the previous day's intake. This ensured that some straw remained in the feed troughs all the time. At each feeding, a solution containing the required amount of urea to supply the estimated RDP requirements of the rumen microbes (ARC, 1984) was thoroughly mixed with straw (300 ml per kg straw) in diets US and US-480. A premix of vitamins and minerals containing sodium sulphate (Table 3.1A) to attain a RDN: S ratio of about 14: 1 (ARC, 1980) was mixed with the straws. Water was freely available.

During both the adjustment and experimental periods straw refusals were collected daily. The refusals for four-day periods were bulked and dried and four-day means for straw and total DMI calculated. For diets US-480 and TS-480, the desired proportion of straw and lucerne was achieved by adjusting the allowance of lucerne pellets every four days according to the previous four-day mean straw intake. Data on feed intake used for the statistical analysis were recorded as detailed in Table 5.1.

5.2.3. Sieving technique for particle size analysis of feed, digesta and faeces

5.2.3.1. Sieving apparatus

Wet sieving of samples was done in a variable-speed sieve shaker (Model EV51, Endecotts Ltd, London). It consisted of a horizontal vibrator encased in a heavy-metal base designed to accommodate a lid, a bank of sieves (20 cm internal diameter) and a base pan and fitted with a screw-clamping device to tighten the lid. Six sieves with apertures (side of a square hole) of 4.75, 2.36, 1.18, 0.60, 0.30 and 0.15 mm (ASAE, 1967; Kennedy, 1984) were arranged vertically, in order of descending aperture size, on top of the base pan. During sieving, a spray of water entering through a nozzle on the lid and running down through the sieves to an evacuating pipe at the base pan washed the sample particles while the vibrator was in motion.

5.2.3.2. Sieving procedure

Samples of faeces and feeds were soaked in water for 12 h to facilitate wet-sieving (Poppi *et al*, 1980). Faeces were broken down very gently by hand to reduce the number of particles from the outer surface of the pellets remaining as conglomerates (Poppi *et al*, 1980). Samples of rumen digesta did not require soaking in water before sieving.

TABLE 5.1 Schedule of the experimental period

Day of experiment	Experimental procedure
1–14	Adjustment period
15	Administration of Cr-mordanted straw (containing small particles) into the rumen at 09:00 h.
15–20	Total collection of faeces 20, 24, 28, 32, 36, 48, 60, 72, 96 and 120 h after administration of the Cr-mordanted straw. Measurement of feed intake.
21	Direct sampling and complete emptying of the rumen of sheep offered diets US and TS, at about 6 h post-feeding.
23–30	Measurement of feed intake
31	First administration of Cr-mordanted straw (containing large and small particles) into the rumen at 09:00 h.
31–36	Total collection of faeces 20, 24, 28, 32, 36, 48, 60, 72, 96 and 120 h after administration of the Cr-mordanted straw. Measurement of feed intake.
37	Second administration of Cr-mordanted straw (containing large and small particles) into the rumen at 9:00h.
37–41	Sampling of rumen digesta 6, 12, 18, 24, 36, 48, 60 and 72 h after the administration of the Cr-mordanted straw.

Before mechanical sieving, all samples were stirred into about 500 ml of water and placed on the top sieve where they were washed with a thin jet of water for about 3 min. This was done to effect a preliminary separation and minimize matting of particles. The lid was then screw-clamped and water sprayed into the apparatus at a predetermined rate, which was sufficient to wash down the sample particles but not to fill the column of sieves. Subsequently, the vibrator was switched on and the horizontal vibration amplitude increased slowly to a maximum of 3 mm, which was maintained for a specific period of time. The particles on each sieve were then carefully washed into a tared polythene basin which was then placed in a forced-draft oven at 100 °C for 24 h. The results were expressed as g DM retained on each sieve per kg of total DM retained on the six sieves. This ratio rather than g DM per kg of total sample DM was adopted in order to reduce variation due to differences in the concetrations of soluble DM in the samples.

The total sieving time and sample size used were selected after studying the effect of various times and sample sizes on the particle size distribution of a pooled sample of digesta, obtained after complete emptying of the rumen of the sheep consuming diets US and TS. Duplicate samples containing 5, 10 and 15 g of DM were sieved for 10, 15, 20 and 25 min. It was assumed that the most appropriate sieving time and sample size selected from results with rumen digesta applied to samples of feed and faeces (See Poppi *et al*, 1980).

A wet-sieving rather than a dry-sieving technique was selected as the results obtained may be more meaningful relative to the physical processes ocurring in the gastrointestinal tract (Allen *et al*, 1984; Uden and Van Soest, 1982). Moreover, the drying of wet samples (digesta and faeces) before dry sieving can induce clustering, shrinkage and breakage of particles (Moseley, 1984).

5.2.4. Determination of rates of particle size reduction in the rumen and rates of escape of particles from this organ

5.2.4.1. Assumptions made

a. Feed particles retained on sieves with aperture size larger than 1.00–1.18 mm have a low probability of passage from the rumen (Poppi *et al*, 1980; Poppi *et al*, 1981c; Poppi *et al*, 1985; Reid *et al*, 1977; Ulyatt *et al*, 1976). In the present experiment, particles retained on sieves of aperture size larger than 1.18 mm are defined as large particles whereas particles passing a 1.18–mm sieve are defined as small particles. The usefulness of this assumption was tested by determining the particle size distribution in duplicate samples of the faeces of sheep on all treatments. The proportion of faecal particles retained on sieves of aperture size larger than 1.18 mm would be very small if the assumption were correct.

b. Large feed particles can disappear from the large–particle pool in the rumen by digestion (k_{dl}), escape to the hindgut (k_{el}) and reduction to small particles (k_r). The total rate of disappearence of large particles from the large–particle pool in the rumen is :

$$k_l = k_{dl} + k_{el} + k_r$$

Small particles can disappear from the rumen by digestion (k_{ds}) and escape (k_{es}). The total rate of disappearance of small particles from the rumen is:

$$k_s = k_{ds} + k_{es}$$

(Poppi *et al*, 1981c; Van Soest, 1982).

c. Feed particles undergo little change in size after leaving the rumen (Grenet, 1970; Poppi *et al*, 1980; Smith *et al*, 1967).

5.2.4.2. Preparation of chromiun-mordanted straw particles

Particles of untreated and treated straw were mordanted with chromium (Cr) according to the method of Uden, Colucci and Van Soest (1980). For each straw, two fractions differing in particle size distribution were prepared for Cr-mordanting. The fractions were a mixture of large and small particles (Cr-LS) and small particles only (Cr-S). To prepare these fractions straw samples were ground in a rotary mill fitted with screens of 8, 5, 3, 2 and 1-mm aperture size to obtain particles of a wide range of size. Equal amounts of samples ground through the 8, 5, 3 and 2-mm screens were mixed with the aim of obtaining a straw fraction containing a large particle: small particle ratio of about 6: 4, which has been observed in the swallowed boluses of sheep fed a wide range of forages (Weston and Kennedy, 1984). The straw sample ground through a 1-mm screen yielded the small-particle fraction.

During the Cr-mordanting process the Cr-LS straw was washed on a 1.18-mm sieve whereas the Cr-S straw was washed on a 20 µ sieve. In all instances (Table 5.1), the Cr-mordanted untreated straws were immersed into the rumen of sheep offered the US and US-480 diets whereas the Cr-mordanted treated straws were immersed into the rumen of sheep offered the TS and TS-480 diets. Before using these Cr-mordanted straws, their particle size distribution and DM disappeareance from dacron bags incubated for 24 h in the rumen of sheep or in a bath with circulating water at 38 °C were determined. Samples of the Cr-mordanted straws were wet-sieved as described previously. The results are shown in Table 5.2. The proportions of particles retained on sieves with aperture size larger than 1.18-mm or passing through a 1.18-mm sieve in

TABLE 5.2 Disappearance of straw DM from dacron bags incubated in the rumen of sheep or in circulating water at 37 °C for 24 h and particle size distribution of Cr-mordanted straws.

Straw type	g DM/kg total DM retained[1] Sieve aperture size, mm		DM disappearance, g/kg Rumen	Water bath
	>1.18	<1.18	Mean±s.e.	Mean±s.e.
Cr-LS[2]				
Untreated	695.6	304.4	-5±0.3	38±1.2
NH_3-treated	686.0	314.0	74±0.3	33±1.1
Cr-S[2]				
Untreated	0	1000	-19±4.8	27±1.1
NH_3-treated	0	1000	34±8.2	27±0.8

1. On six sieves with aperture sizes of 4.75, 2.36, 1.18, 0.6, 0.3 and 0.15 mm.
2. See text.

the Cr-LS untreated straw were very similar to those in the Cr-LS treated straw. The large particle: small particle ratios in the Cr-mordanted straws were very close to the intended ratio of 6:4 . The disappearance of straw DM from dacron bags incubated in the rumen and in a water bath were small. The small differences between the values for the bags incubated in the rumen and in the water bath indicated that the DM lost was mostly particles escaping through the bag pores and perhaps some unbound Cr. The Cr-mordanted straws were therefore considered undegradable.

5.2.4.3. Determination of rates of size reduction of large straw particles in the rumen

a. Sampling of rumen digesta

The need for appropriate methods to obtain representative samples of intact rumen contents and hence accurate estimates of the rates of particle size reduction in the rumen has been recognized (Mertens, Strawn and Cardoza, 1984). Thus, a simple instrument to sample rumen digesta was developed and tested during the course of the present experiment. It consisted of a pair of laboratory tongs (45 cm long) with a metal scoop welded by its convex surface on the inner side of each gripping end of the tongs. The overall width of the tongs was reduced so that they could be inserted and manipulated through the 4-cm rumen cannulae of the sheep. At each sampling, the tongs were kept closed while being introduced through the rumen cannula and directed to the desired sampling site. The tongs were then opened and turned 90 ° about their axis. After closing the tongs, a sample was tightly grabbed by the scoops and transferred to a storing container.

The efficacy of the sampling procedure for rumen digesta was tested by comparing the DM concentration and the particle size distribution of digesta

samples taken directly from the rumen with the modified tongs or taken from the whole contents which had been removed from the rumen through the cannula. The sheep consuming diets US and TS were used for these comparisons. At about 6 h post-feeding on day 21 of the experiment (Table 5.1), the rumen digesta of each sheep was sampled as described previously. Each sample was composed of twelve subsamples taken from different sites of the reticulo-rumen: one towards the anterior portion, two from the middle portion and one towards the caudal portion at each of three vertical levels, namely, dorsal, middle and ventral. Immediately after sampling, most of the fluid-rich fraction of the rumen contents was removed with a hand vacuum pump. The particle-rich fraction left in the rumen was removed through the cannula with the modified tongs. Both fractions were then mixed in a large container and subsamples taken. Duplicate samples of rumen digesta obtained by both methods were analyzed for DM concentration and particle size distribution. The wet-sieving technique used for particle size analysis of these samples was as described previously.

b. Rates of size reduction of large straw particles

On day 37 of the experiment (Table 5.1), a tissue-paper pellet, containing 75 g DM of the Cr-LS straws, was immersed in the rumen of all the sheep. Samples of rumen digesta were taken 6, 12, 18, 24, 36, 48, 60 and 72 h after immersing the Cr-LS straws into the rumen. These samples were frozen before being separated into large and small particles by wet-sieving. The Cr concentration in the large-particle fraction was determined as described in Appendix 4.1A . When the total weight (DM) of the large particle fraction obtained from sieving samples of rumen digesta was less than the 3 g specified in Appendix 4.1A, Cr determinations were done with the total amount of sample available.

To calculate the rates of size reduction of large straw particles in the rumen, it was assumed that the flow of particles from the large-particle pool was proportional to the size of the pool. The uselfulness of this assumption depends on whether the size of the large-particle pool remains approximately constant with time (Blaxter, Graham and Wainman, 1956; Hungate, 1966; Mertens *et al*, 1984) The size of this "pool" was not estimated in the present trial, but it was observed that the concentration of large particles in samples of rumen digesta varied according to sampling time (Figure 5.1). In an attempt to reduce the effect of this variation on the estimation of k_r, the concentrations of Cr in the large-particle fractions of samples of rumen digesta were standarized to a constant proportion of large particles for each sheep. The Cr concentration of a sample from a particular time was expressed as mg Cr per kg of large-particles DM multiplied by the following ratio:

$$\frac{\text{g large-particles DM/kg total-sample DM at a particular time}}{\text{Average of g large-particles DM/kg total-sample DM}}$$

Where the average proportion large-particles was calculated using data for all sampling times for each sheep. The plot of the natural logarithm of the standarized Cr concentrations showed a linear decrease with time (Figure 5.2).

The coefficient of the linear regression fitted to these data was defined as the rate of disappearance of Cr-mordanted large particles from the large-particle pool in the rumen (k_l). Following Section 5.2.4.1 (Assumption b), k_l is equal to the sum of the rates of digestion (k_{dl}), escape to the hindgut (k_{el}) and reduction to small particles (k_r). Since the Cr-mordanted straws were found to be undegradable (Table 5.2), k_{dl} was assumed to be equal to zero. The rate of escape of large particles from the rumen to the hindgut (k_{el}) was expected to be very small (See Section 5.2.4.1). Therefore, the coefficient of the linear

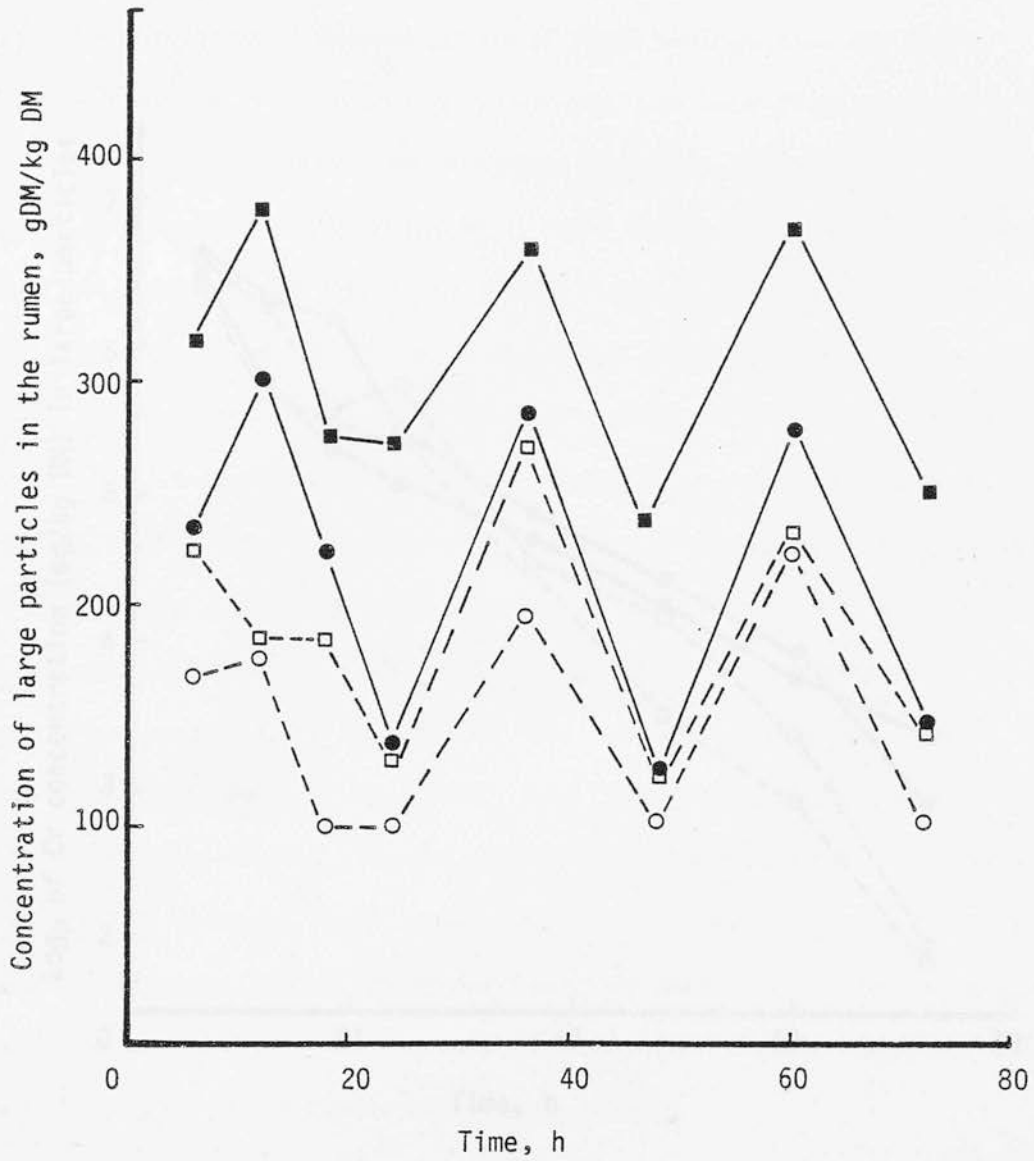

FIGURE 5.1 Changes in concentration of large particles in the rumen with time after the administration of Cr-mordanted straw into the rumen of sheep offered diets: US (● —— ●); US-480 (o---o); TS (■——■) and TS-480 (□---□). (Examples of one sheep from each diet).

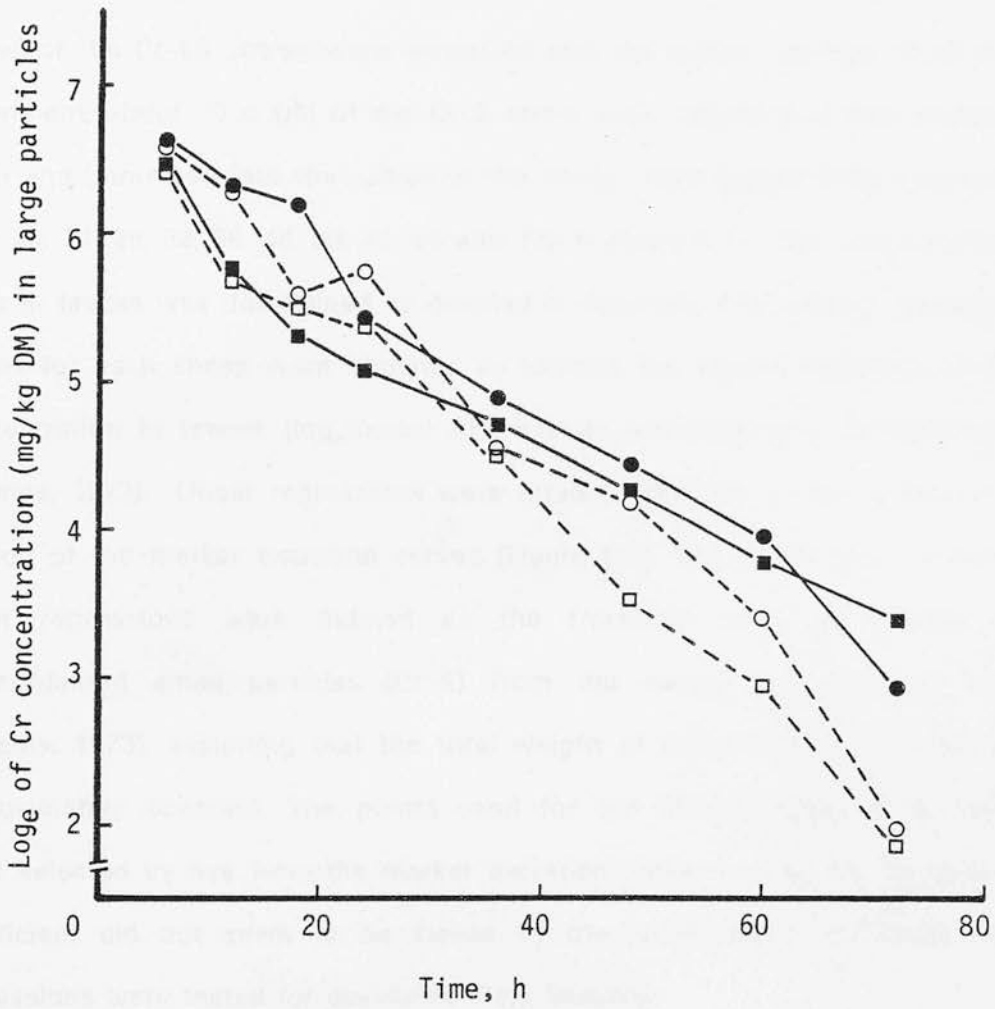

FIGURE 5.2 Changes in $\log_e$ of chromium (Cr) concentration in large particles from rumen digesta with time after administration of Cr-mordanted straw into the rumen of sheep offered diets: US (●—●); US-480 (○--○); TS (■—■) and TS-480 (□--□). (Examples of one sheep on each diet).

regression of the $\log_e$ of the standarized Cr concentration in large particles on time (Figure 5.2) was also defined as k_r (*i.e.* $k_l = k_r$).

5.2.4.4. Determination of rates of escape of straw particles from the rumen

The rate of escape of particles from the rumen was determined after the Cr-S straws or the Cr-LS straws were immersed into the rumen. On day 15 of the experiment, about 35 g DM of the Cr-S straw were packed in a tissue-paper pellet and immersed into the rumen of the sheep. Total faeces were collected after 20, 24, 28, 32, 36, 48, 60, 72, 96 and 120 h (Table 5.1). The concentration of Cr in faeces was determined as detailed in Appendix 4.1A. Marker excretion curves for each sheep were obtained by plotting the natural logarithm of Cr concentration in faeces ($\log_e$[faecal Cr]) against sampling time (Grovum and Williams, 1973). Linear regressions were fitted to the data in the descending portion of the marker excretion curves (Figure 5.3). The coefficients of these linear regressions were defined as the fractional rates of escape of Cr-mordanted small particles (Cr-S) from the rumen (k_{es}) (Grovum and Williams, 1973), assuming that the total weight of rumen contents remained approximately constant. The points used for the linear regression analysis were selected by eye from the marker excretion curve so that the regression coefficient did not seem to be biased by the points about the peak. All regressions were tested for deviations from linearity.

On day 31 of the experiment about 35 g DM of the Cr-LS straws were packed in a tissue-paper pellet and immersed into the rumen of the sheep. Collection of faeces, analysis of Cr and calculation of the rates of escape of Cr-mordanted particles from the rumen were done as described above for Cr-S straws. The coefficients of the linear regressions fitted to the data in the descending portion of the marker excretion curves were defined as the

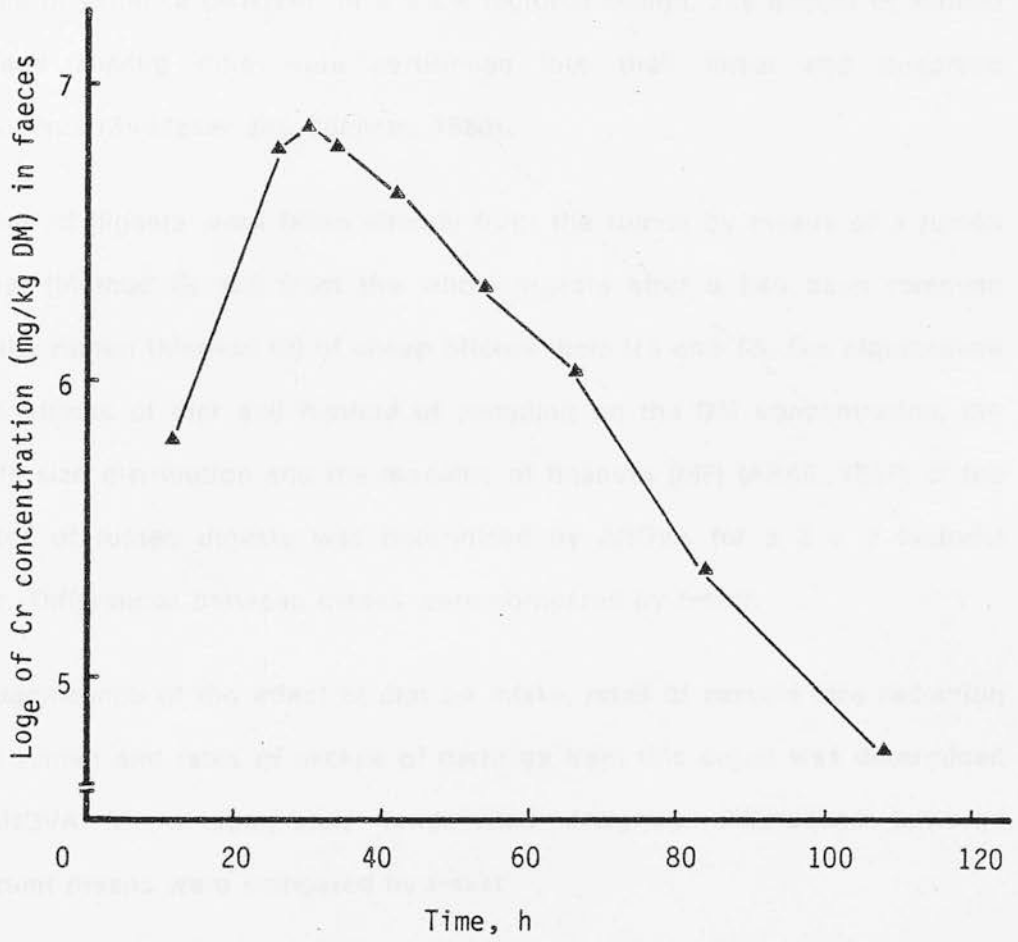

FIGURE 5.3 Changes in $\log_e$ of chromium (Cr) concentration in faeces with time after administration of Cr-mordanted straw into the rumen of a sheep offered untreated barley straw.

fractional rates of escape of Cr-mordanted large and small particles (Cr-LS).

5.2.5. Statistical analyses

The significance of the effects of sample size (5, 10 and 15 g) and sieving time (10, 15, 20 and 25 min) on the particle size distribution of a pooled sample of rumen digesta from sheep offered diets US and TS was determined by analysis of variance (ANOVA) for a 3 x 4 factorial design. The effects of sample size and sieving time were partitioned into their linear and quadratic components (Snedecor and Cochran, 1980).

Samples of digesta were taken directly from the rumen by means of a rumen sampler (Method S) and from the whole digesta after it had been removed from the rumen (Method W) of sheep offered diets US and TS. The significance of the effects of diet and method of sampling on the DM concentration, the particle size distribution and the modulus of fineness (MF) (ASAE, 1967) of the samples of rumen digesta was determined by ANOVA for a 2 x 2 factorial design. Differences between means were compared by t-test.

The significance of the effect of diet on intake, rates of particle size reduction in the rumen and rates of escape of particles from this organ was determined by ANOVA for a completely randomized designed. Differences between treatment means were compared by t-test.

5.3. RESULTS

5.3.1. The effect of sample size and sieving time on the particle size distribution in rumen digesta

Means of the different size-fractions of rumen digesta according to sample size and sieving time are shown in Table 5.3 . A summary of the ANOVAs for each fraction is given in Table 5.1A. The total proportion of particles retained

TABLE 5.3 Effect of sample size and sieving time on the particle size distribution (g DM/total DM retained on sieves) of rumen digesta from sheep offered straw-based diets.

Sieve aperture size, mm	Sample size, g	Sieving time, min			
		10	15	20	25
>1.18	5	303	319	325	262
	10	286	280	257	286
	15	292	271	265	262
4.75	5	98	117	119	98
	10	137	146	132	152
	15	153	164	170	135
2.36	5	65	73	72	46
	10	45	56	44	40
	15	51	40	44	47
1.18	5	140	129	134	117
	10	103	78	81	94
	15	88	68	51	80
0.60	5	130	123	133	128
	10	162	187	143	149
	15	195	202	176	177
0.30	5	321	278	269	334
	10	284	291	309	312
	15	279	234	321	307
0.15	5	246	280	273	276
	10	267	241	290	253
	15	233	292	238	253

on sieves with aperture size larger than 1.18 mm was not significantly affected ($P<0.05$) by sample size or sieving time. The effect of sieving time was not significant ($P<0.05$) for all particle fractions retained on individual sieves, except for the fraction retained on a 1.18-mm sieve. The effect of sieving time on the fraction retained on the 1.18-mm sieve varied with different sample sizes; the effect was linear for the 5 g samples whereas it was quadratic for both the 10 and the 15-g samples. As the sample size increased, the proportions of particles retained on the 4.75 and 0.60-mm sieves increased linearly ($P<0.05$) while the proportions of particles retained on the 2.36 and 1.18-mm sieves decreased linearly ($P<0.05$ and $P<0.001$, respectively). The proportions of particles retained on sieves of aperture size of 0.30 and 0.15 mm were not significantly affected by sample size or sieving time ($P<0.05$). Taking these results into consideration it was decided to separate rumen digesta into large and small particles for determinations of the rates of particle size reduction (See Section 5.2.4.3.b) by sieving samples containing 10 g DM for 20 min. These values were selected for the following reasons:

a. The amount of large particles (DM) obtained after sieving samples of rumen digesta containing 5 g DM was not sufficient for accurate analysis of Cr.

b. With samples containing 15 g DM, large particles tended to mat on the top sieve, as indicated by the larger proportion of particles retained on the 4.75-mm sieve with 15 g DM samples compared to smaller samples (Table 5.3).

c. Generally, the proportions of particles retained on the different sieves tended to stabilize toward 20-25 min. On average, the differences between the means for the 25 and 20-min sieving times were the smallest of all differences between the means for the other sampling times.

For reasons b. and c. above, a sample size of 10 g DM and a sieving time of

20 min were also adopted for the particle size analysis of faeces and Cr-mordanted straws.

The repeatibility of the wet-sieving method for separation of samples into large and small particles was examined by sieving four samples of rumen digesta from sheep offered diet US and four samples from sheep offered diet TS. The rumen digesta was a pooled sample from three sheep per diet. The sieving technique was as described in Section 5.2.3.6. The means for the concentrations of large particles (g DM per kg total sample DM) in rumen digesta of sheep offered diets US and TS were 296 (CV=0.058) and 449 (CV=0.036), respectively.

5.3.2. Particle size distribution in faeces

A small fraction (10-70 g per kg) of the particulate DM in faeces was retained on sieves of aperture size larger than 1.18 mm (Figure 5.4). This fraction of large particles was lower in diet US than in diets US-480 and TS, which did not differ between them. It was similar between diet TS-480 and the other three diets (Figure 5.4 and Table 5.3A).

5.3.3. Sampling of rumen digesta

The DM concentration, the particle size distribution and the MF of samples of digesta taken directly from the rumen with the sampler (Method S) were very similar to those of samples taken from the whole digesta after it had been removed from the rumen (Method W) (Tables 5.4 and 5.2A). A significant ($P<0.01$) difference between methods S and W was detected only for the proportion of particles retained on a 1.18-mm sieve.

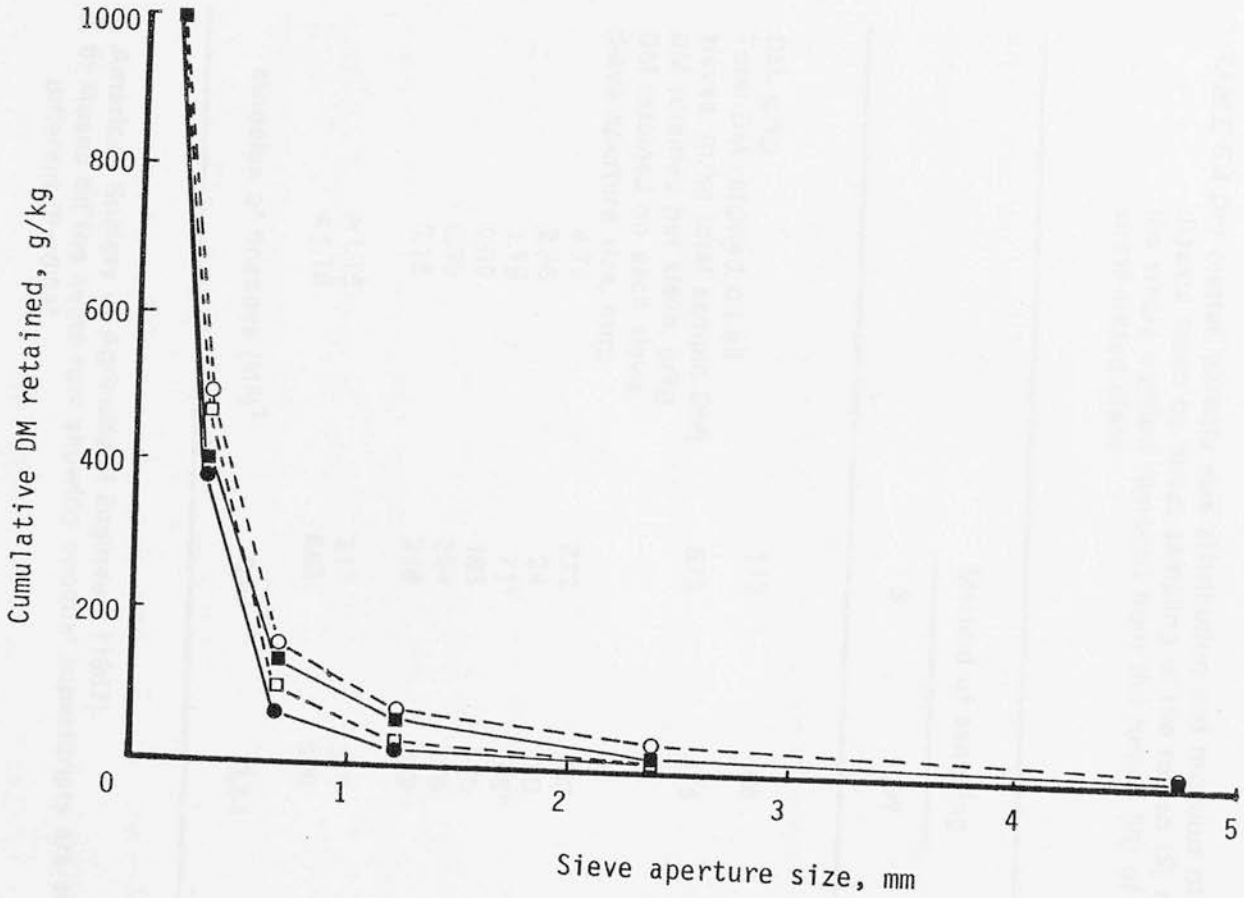

FIGURE 5.4 Cumulative DM retained on sieves of various aperture sizes for faecal samples taken from sheep offered diets US (●——●); US-480 (o---o); TS (■——■) and TS-480 (□---□).

TABLE 5.4 Dry matter, particle size distribution and modulus of fineness of digesta taken by direct sampling of the rumen (S) or by sampling the whole content removed from the rumen (W) of sheep offered straw-based diets.

	Method of sampling		
	S	W	s.e. diff.
DM, g/kg	113	98	7.0
Total DM retained on all sieves. (g/kg total sample DM)	573	575	21.0
DM retained per sieve, g/kg			
DM retained on each sieve:			
Sieve aperture size, mm:			
4.75	222	183	42.3
2.36	24	29	7.5
1.18	71[a]	98[b]	7.5
0.60	183	175	12.4
0.30	254	276	19.8
0.15	246	239	24.1
>1.18	317	310	39.6
<1.18	683	690	39.6
Modulus of fineness (MF)[1]	3.04	2.94	0.18

1. American Society of Agricultural Engineers (1967).
a, b: Means on the same row showing unequal superscripts are significantly different ($P<0.01$).

5.3.4. Feed intake, rate of size reduction of large particles in the rumen and rate of escape of particles from this organ

The straw OMI of sheep eating diet US-480 was significantly lower ($P<0.05$) than the straw OMI by sheep eating diets US, TS and TS-480 which did not differ from each other ($P<0.05$) (Table 5.5). Surprisingly, the intake of straw in diets US and TS were not significantly different ($P<0.05$). Total OMI was highest for diet TS-480 followed by diets TS, US-480 and US.

The rates of size reduction of large straw particles in the rumen (k_r) were similar for all diets ($P<0.05$) (Table 5.5), though the mean for diet US was higher than the means for the other three diets. The rate of escape of small straw particles from the rumen (k_{es}) was faster in sheep fed diet TS-480 compared to the other three diets which did not differ from each other ($P<0.05$). The rate of escape of large and small particles (k_{els}) was lower ($P<0.1$) for diet US-480 than for the other three diets which had similar values ($P<0.05$). A summary of the ANOVAs for the variables in Table 5.5 is given in Table 5.3A.

Generally, the marker excretion curves used to determine k_{es} and K_{els} did not show a sharp division between the ascending and the descending portion. Moreover, the deviations from linearity (significance of a quadratic component) were significant ($P<0.05$) for most regressions calculated with the data in the descending portion,especially for the marker excretion curves used to estimate k_{es}. Examples of excretion curves for one sheep on each treatment which illustrate this more clearly are shown in Figure 5.5. For the estimation of k_{els} it was considered unneccessary to determine the Cr-concentration of faecal samples collected 20 h after the administration of the Cr-LS straw. The excretion curves of Cr when Cr-S straw was immersed into the rumen almost

TABLE 5.5 Intake, rate of size reduction of large straw particles (k_r), rate of escape of small straw particles (k_{es}) and rate of escape of large and small straw particles (k_{els}) from the rumen of sheep offered straw-based diets.

	Diet description				s.e. diff
	US	US-480	TS	TS-480	
Daily intake					
g OM per kg $LW^{0.75}$					
Straw	37.9^{a}	23.2^{b}	42.4^{a}	36.5^{a}	4.8
Total	37.9^{a}	42.2^{a}	42.4^{a}	66.2^{b}	5.4
k_r, per h	0.062	0.046	0.055	0.057	0.0127
k_{es}, per h	0.023^{da}	0.019^{a}	0.020^{a}	0.033^{be}	0.0045
k_{els}, per h	0.019^{de}	0.011^{e}	0.018^{de}	0.026^{d}	0.0057

Means with different superscripts in the same row are significantly different; a, b, c : $P<0.05$; d, e : $P<0.1$.

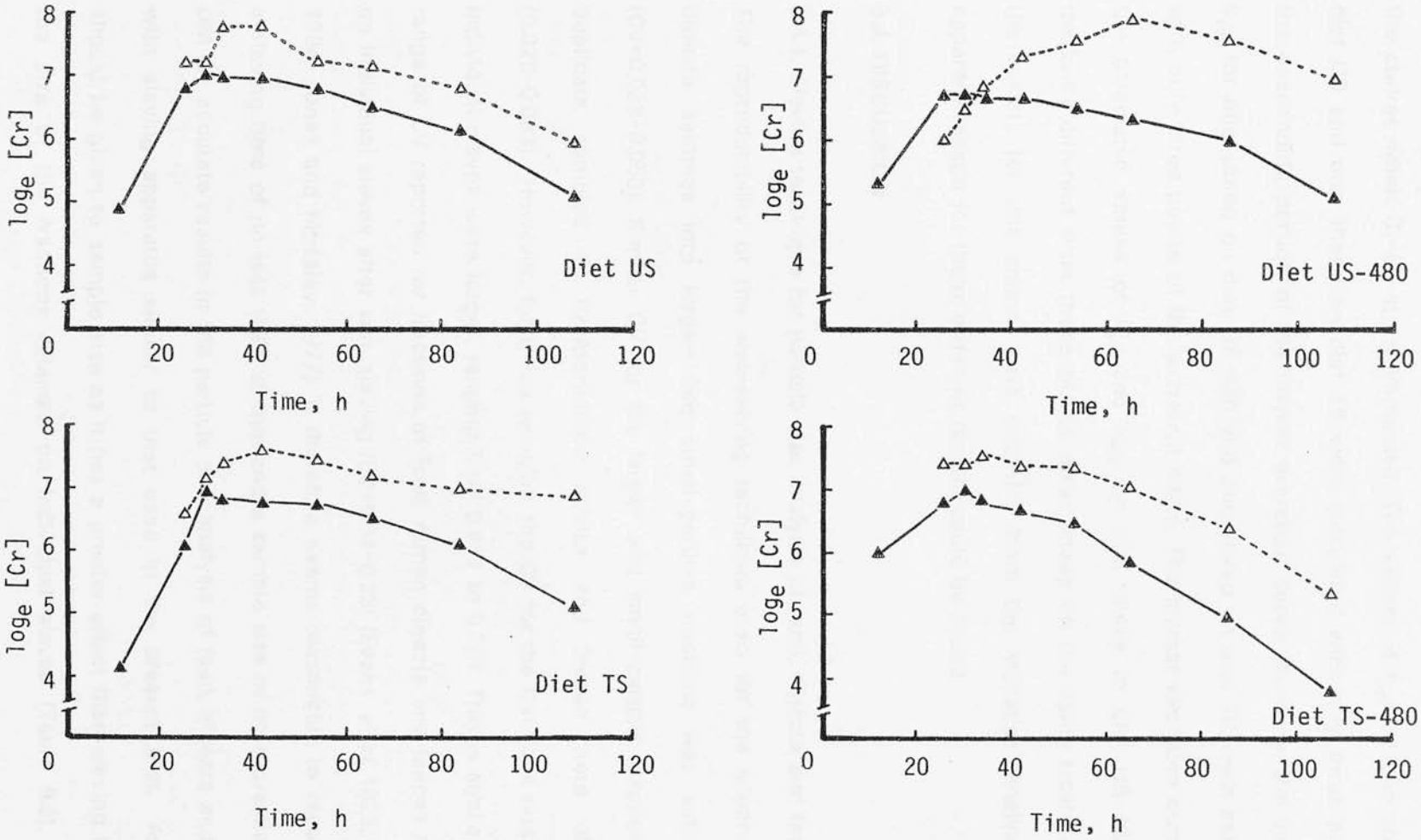

FIGURE 5.5 Changes in the $\log_e$ of chromium concentration (mg/kg DM) ($\log_e$ [Cr])in faeces with time after administration of Cr-mordanted straws containing large and small particles (△---△) or small particles (▲—▲) into the rumen of sheep offered straw-lucerne diets.

invariably reached a peak sooner and descended at a slighlty faster rate than the curves when Cr-LS straw immersed. The values of k_{es} for one sheep on diet US and one sheep on diet TS were calculated with only three points in the descending portion of the marker excretion curve. Similarly, the values of k_{els} for one sheep on diet US-480 and one sheep on diet TS were calculated with only three points of the excretion curve. The marker excretion curves and the calculated values of k_{es} and k_{els} for one sheep in diet US-480 were markedly different from those of the other sheep on the same treatment and the results for this animal were excluded from the statistical analysis. No apparent reason for these different results could be found.

5.4. DISCUSSION

5.4.1. Sieving technique for particle size analysis of feed, digesta and faeces

The reproducibility of the wet-sieving technique used for the separation of digesta samples into large- and small-particle fractions was satisfactory (CV=0.028-0.058). Similar CV for the large- and small-particle fractions from duplicate samples of Cr-mordanted straws and faeces were observed (0.025-0.083). However, for these samples, the CV for the fractions retained on individual sieves were larger, ranging from 0.016 to 0.281. This is similar to the range of CV reported for fractions of feed, rumen digesta and faeces retained on individual sieves after wet sieving (CV=0.04-0.23) (Evans *et al*, 1973; Grenet, 1984; Jones and Moseley, 1977). It therefore seems reasonable to recommend a sieving time of no less than 20 min and a sample size of no more than 10 g DM for accurate results in the particle size analysis of feed, digesta and faeces with sieving apparatus similar to that used in the present trial. Attention should be given to sample size as it has a greater effect than sieving time on the size of the fractions retained on individual sieves (Table 5.3). Similar

results were observed by Jones and Moseley (1977) and Moseley (1984). A low sample size:sieve area ratio is important to minimize matting of particles on the top sieves and blocking of the water flow with wet-sieving methods (See Jones and Moseley, 1977). In the present trial, this ratio for the 10 g DM samples was 0.03 g DM per cm^2. This is within the range of values in reports where good repeatibility of the wet-sieving technique and minimum matting of particles were observed (Allen *et al*, 1984; Grenet, 1984; Jones and Moseley, 1977; Moseley, 1984; See Table 2.1A). Moreover, the preliminary separation of particles by washing the samples before mechanical sieving was found to be very effective to minimize matting on the top sieves. Such a preliminary washing may be more important when sieving apparatus with horizontal vibration are used.

5.4.2. Sampling of rumen digesta

An important finding of the present work is that representative samples of the rumen contents were obtained by means of a simple and inexpensive instrument (rumen sampler). The samples obtained were representative in terms of DM concentration and particle size distribution. Whether they had a chemical composition similar to that in the whole rumen digesta was not examined. Moreover, the rumen sampler was tested for digesta taken 6 h post-feeding in sheep fed straw diets *ad libitum* and twice daily. It would be interesting to test the rumen sampler over a wider range of roughage-based diets, levels of feeding and times after feeding because it is simple to make and easy to use with sheep having small rumen cannulae. With the instrument used here, the method of sampling rumen digesta is simpler than that proposed by Egan, Pearce, Doyle and Thomas (1983) for sheep fed chopped roughages. Moreover, indirect measurements of the quantity and composition of the rumen digesta carried out by sampling via a fistula are less

expensive and cumbersome than direct methods such as slaughtering (Warner, 1981) and complete emptying of the rumen through a large cannula (*e.g.* Poppi *et al*, 1981a).

5.4.3. Feed intake

Surprisingly, the intake of treated straw was very similar to the intake of untreated straw. An increase in straw OMI due to ammonia-treatment would have been expected since the *in vivo* OMD of the untreated and the treated straw used were 0.512 and 0.603, respectively (See Chapter 4). However, no definitive conclusions can be drawn from the intake results in the present trial because intake was very variable among the three sheep on each diet (CV=0.05-0.21). Although it is generally accepted that ammonia-treatment increases the intake of cereal straws, the responses reported have been very variable partly because of differences in the level and type of other feeds given with the straws, the physical form of the diet and the animal species (Abidin and Kempton, 1981; Al-Rabbat and Heaney, 1978a; Dryden and Kempton, 1983; Horton, 1978; Horton and Steacy, 1979; Lawlor and O'Shea, 1979). There are few reported experiments where the effect of ammonia-treatment on the intake and digestibility of chopped cereal straws has been studied with sheep fed the straws alone or supplemented with urea and/or minerals and vitamins only. In the present trial, the increase in straw OMI due to ammonia-treatment was small (0.12 of the intake of untreated straw). This was larger than the increase of 0.04 reported by Abidin and Kempton (1981) for sheep fed chopped barley straw representing about 0.8 of the total diet but, it was smaller than the increase of about 0.24 reported by Dryden and Kempton (1983) for sheep fed chopped barley straw *ad libitum* and urea and/or a mineral supplement. The straw in the present work was oven-treated whereas the straws in the experiments of Abidin and Kempton

(1981) and Dryden and Kempton (1983) were stack-treated. However, this does not necessarily mean that the intake responses in the present trial should have been expected to be higher because oven treatment is not always more efficient than stack treatment for up-grading the feeding value of cereal straws (Ibbotson, Mansbridge and Adamson, 1984; Sundstøl and Coxworth, 1984).

5.4.4. Rates of size reduction of large particles in the rumen and rates of escape of particles from this organ

5.4.4.1. Assumptions made

The first assumption made to calculate rates of size reduction of feed particles in the rumen (k_r) was that particles retained on sieves with aperture size larger than 1.18-mm represented those particles in the rumen that require further breakdown before they can pass to the omasum. Accordingly, feed particles passing through a 1.18-mm sieve were assumed to represent those particles that could leave the rumen without further comminution. The assumption made was based on the fact that several workers have found that very few particles in post-ruminal digesta and faeces are retained on sieves of aperture size larger than 1.00-1.18 mm (Pearce, 1967; Poppi *et al*, 1981c; Reid *et al*,1977; Troelsen and Campbell, 1968; Ulyatt *et al*, 1976). Similar results were found in the present experiment. In all diets, the proportion of faecal particles retained on sieves with aperture size larger than 1.18 mm was 0.01-0.07 of the total faecal particulate matter (Figure 5.4). The proportions of large particles in faeces differed among diets, but no apparent explanation for the trends observed could be found. Moreover, the large-particle fraction was so small that it was not accurately measured (within-diet CV ranged from 0.27 to 0.89).

A second assumption made when calculating k_r was that the rate of escape of

large particles from the rumen (k_{el}) was equal to zero. The validity of this assumption can be supported with the results of the particle size distribution in faeces showing a very low proportion of large particles. Moreover, Weston and Cantle (1984) showed that large particles of straw fibre (retained on a 1.2-mm sieve) escape from the rumen at a negligible rate (0.001 per h).

A third assumption made to calculate k_r was that the size of the large-particle pool in the rumen was approximately constant. The size of this pool was not estimated in this trial, but the proportion of large particles in rumen digesta varied according to sampling time (Figure 5.1). Therefore, the size of the large-particle pool would have changed with time even if the total rumen volume had had remained approximately constant. In an attempt to reduce the effects of such a variation on the values of k_r, the Cr concentrations in all samples of large particles were standarized to a constant proportion of large particles in the rumen (Section 5.2.4.3 b.). As a result, the coefficients of determination (r^2) of the linear regression of $\log_e$(Cr concentration) on time (Figure 5.2) were markedly increased compared to the r^2 of linear regressions with unstandarized Cr concentrations. It was unfortunate that feeding in the present trial could not be done more frequently than twice daily so that rumen conditions closer to a steady state could have been achieved. However, the straw was offered *ad libitum* with a large margin for refusals aiming at reducing changes with time in rumen volume and the size of the large-particle pool. Rumen volume was not measured in the present trial, but it may have changed with time within a relatively narrow range despite large changes in the rumen DM concentration (Hungate, 1966; Ørskov and McDonald, 1979). There is evidence in the literature to suggest that the assumption of a constant rumen volume may not have introduced major errors in the estimation of k_r and the rates of escape of particles from the rumen (k_{es}, k_{els}).

Firstly, Evans *et al* (1973) indicated that their estimates of k_r in cattle fed hay once daily were little affected by changes in rumen volume with time. Secondly, in the work of Milne, MacRae, Spence and Wilson (1978) with sheep fed low-quality roughages twice daily, changes in rumen volume with time were expected to occur. This, however, did not seem to introduce major errors in the estimation of the fractional flow rates of digesta from the rumen (k_1) and from the caecum-proximal colon (k_2), determined by compartmental analysis of the curves of excretion of a marker in faeces. It can be assumed that k_1 and k_2 were reasonably accurate estimates because the sum of their reciprocals, *i.e.* $1/k_1 + 1/k_2$ (which corresponds to the mean retention time of digesta in the total alimentary tract, MRT), was very similar to the MRT determined directly by total recovery in the faeces of a marker given as a single pulse dose. Moreover, in the work of Milne *et al* (1978), k_1 was probably a satisfactory estimate of the fractional flow rate of digesta from the rumen (See Warner, 1981). For the same reasons discussed above, it can be suggested that in the work of Uden *et al* (1982), changes in rumen volume with time did not introduce major errors in the estimation of fractional flow rates of Cr-mordanted fibre from the rumen of sheep fed a medium-quality hay three times daily. Thirdly, in the model simulations of Baldwin *et al* (1977), fractional flow rates of digesta from the rumen estimated with frequent feeding were applicable to infrequent feeding conditions. The reverse may be also true, although it requires systematic testing.

5.4.4.2. Rates of particle size reduction and rates of escape

Rates of size reduction of large particles in the rumen (k_r) varied within a wide range among the sheep in the experiment (0.027-0.075 per h, overall CV=0.27). The k_r obtained here are difficult to compare with published results because it seems that values truly representing k_r have not been reported. As discussed

in the review of literature (Section 2.5.2.1), the values reported as k_r are either the sum of the rate of particle size reduction (k_r), the rate of digestion (k_d) and the rate of escape from the rumen (k_e) or the sum of k_r and k_d. However, the results obtained here are similar to most values in Table 2.2 of the literature review (0.040–0.088 per h, excluding the value reported by Lascano (1979) (Cited by Van Soest, 1982) where $k_d + k_r = 0.156$ per h). The experimental procedures between the experiments summarized in Table 2.2 and the present trial are too different to elaborate valid arguments when making comparisons. A common feature to all experiments in Table 2.2 and to the present experiment is that the rates of particle size reduction are generally faster (0.030–0.088 per h) than reported values for rates of escape of small forage particles from the rumen; *e.g.* 0.018 per h for straw particles passing a 0.6–mm sieve but retained on a 0.3–mm sieve (Weston and Cantle, 1984), 0.026 per h for hay particles passing through a 0.6–mm sieve (Evans *et al*, 1973) and 0.01–0.04 per h for grass particles passing through a 1.18–mm sieve (Poppi *et al*, 1981c).

Surprisingly, k_r values were similar for all diets (Table 5.5). This may have been a real effect but, it may have also been due to the influence of the high variation in k_r on the probability of not detecting significant differences (Snedecor and Cochran, 1980). With the results of the present trial it is not possible to say whether differences in straw intake among diets were associated with differences in k_r because the straws were fed *ad libitum*. Irrespective of the diet, the size of the large–particle pool in the rumen would have been expected to increase as straw intake increased. If the flow of large particles from this pool were proportional to the size of the pool, a good correlation between k_r and straw intake would be expected (r=0.70, Figure 5.6a). The present results are not sufficient to prove the generally accepted

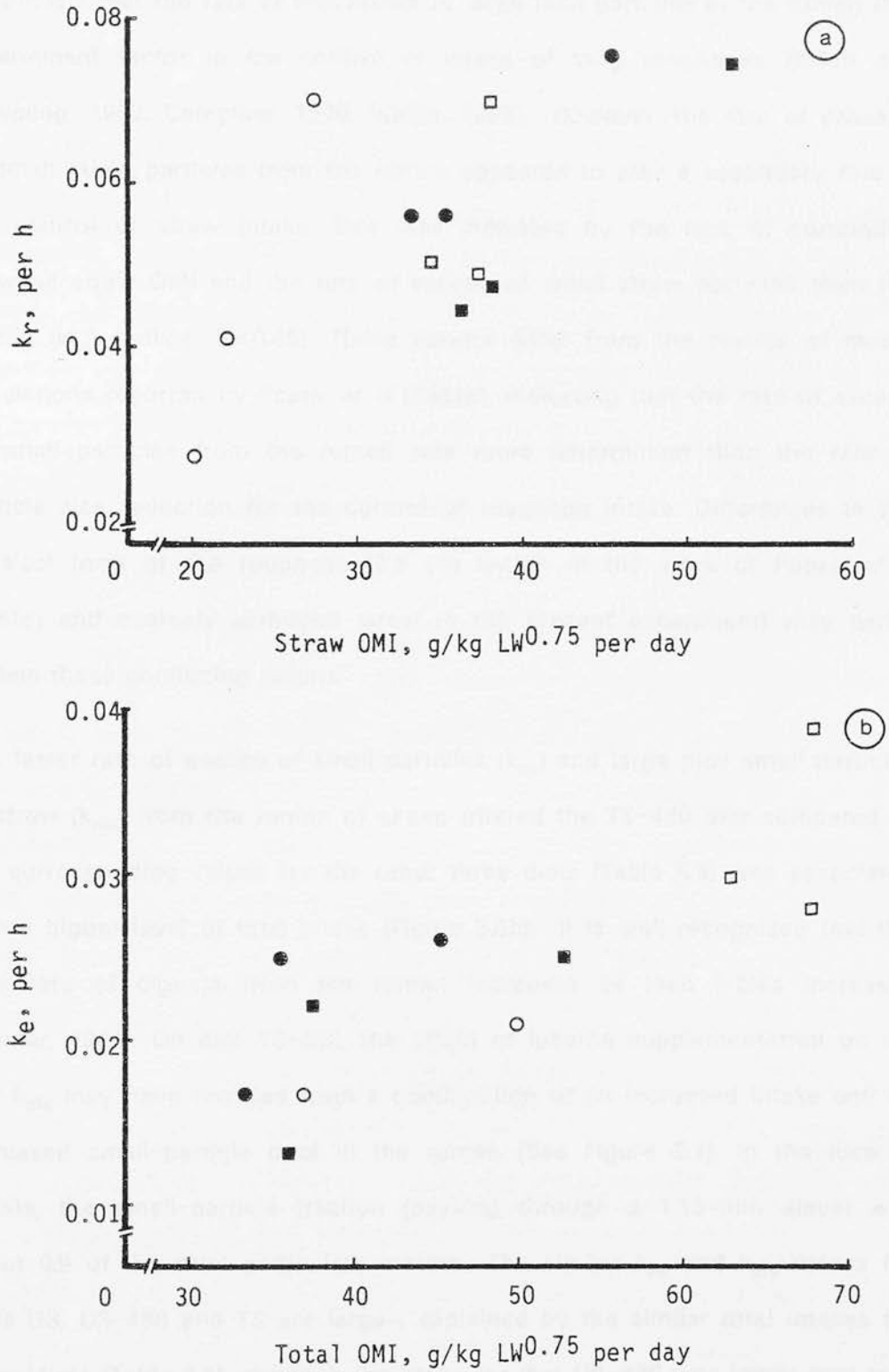

FIGURE 5.6 Relationships between: (a) rate of size reduction of large straw particles in the rumen (k_r) and straw OMI; (b) rate of escape of small straw particles from the rumen (k_e) and total OMI. Diet US (●), US-480 (○), TS (■) and TS-480 (□).

hypothesis that the rate of breakdown of large feed particles in the rumen is a determinant factor in the control of intake of long roughages (Balch and Campling, 1962; Campling, 1970; Welch, 1982). However, the rate of passage of small straw particles from the rumen appeared to play a secondary rôle in the control of straw intake. This was indicated by the lack of correlation between straw OMI and the rate of escape of small straw particles from the rumen (k_{es}) ($r=0.34$, $P<0.05$). These results differ from the results of model simulations reported by Poppi *et al* (1981c), indicating that the rate of escape of small particles from the rumen was more determinant than the rate of particle size reduction for the control of roughage intake. Differences in the physical form of the roughage (2.5 cm length in the work of Poppi *et al* (1981c) and coarsely shredded straw in the present experiment) may partly explain these conflicting results.

The faster rate of escape of small particles (k_{es}) and large plus small particles of straw (k_{els}) from the rumen of sheep offered the TS-480 diet compared to the corresponding values for the other three diets (Table 5.5) was associated with a higher level of total intake (Figure 5.6b). It is well recognized that the flow rate of digesta from the rumen increases as feed intake increases (Warner, 1981). On diet TS-480, the effect of lucerne supplementation on k_{es} and k_{els} may have resulted from a combination of an increased intake and an increased small-particle pool in the rumen (See Figure 5.1). In the lucerne pellets, the small-particle fraction (passing through a 1.18-mm sieve) was about 0.9 of the total particulate matter. The similar k_{es} and k_{els} values for diets US, US-480 and TS are largely explained by the similar total intakes for those diets (Table 5.5), although the mean for diet US-480 was lower than that for diets US and TS. However, it is emphasized that the values cf k_{es} and k_{els} for some sheep were not estimated very accurately and these results should

be interpreted with caution.

In experiment 5 (Chapter 4), with ammonia-treated straw, the *in vivo* digestibility of the diets decreased linearly as the level of lucerne pellets increased from 0 (diet TS-0L) to 480 g DM per kg of total dietary DM (diet TS-480L). It was suggested that such a decrease was probably associated with a faster rate of passage of digesta from the rumen. This suggestion can be supported with the results obtained here with the same feeds; *i.e.* the k_{es} and k_{els} for diet TS-480 were greater than those for diet TS.

Most of the marker excretion curves obtained after administration of the Cr-LS and the Cr-S straws via the rumen cannula did not show a clear definition between the ascending and the descending portion. Moreover, most regressions fitted to the data in these descending portions showed significant deviations from linearity. The k_{es} and k_{els} values, estimated as proposed by Grovum and Williams (1973), were somehow biased as these rates appeared to be changing with time (Figure 5.5). Similar results were reported by Uden *et al* (1982) for Cr-mordanted fibre and by Milne *et al* (1978) for a particulate-phase marker. In both cases, roughages were fed and the two-compartment model proposed by Grovum and Williams (1973) to calculate the flow rate of digesta from the rumen (descending portion of the curve) and from the caecum-colon (ascending portion of the curve) was inappropriate for the results. There were not enough data in the present work to test other models with three pools (Milne *et al*, 1978) or time-dependent rates (Ellis, Matis and Lascano, 1979 (Cited by Van Soest, 1982)), but it is likely that the three-compartmental model would have been a good description of the data. More critical research is needed to know which section of the digestive tract the third pool or compartment may represent and whether the rates of escape of fibre particles

from the rumen are truly time-dependent rather than volume- or mass-dependent.

5.5 CONCLUSIONS

With sheep fed shredded straw-based diets *ad libitum* and twice daily:

a. The particle size analysis of digesta and faeces can be determined accurately by a wet-sieving technique provided that the following conditions are fulfilled:

   - pre-soaking of dry or compact samples for at least 12 h before sieving
   - low sample size:sieve area ratio. Preferably no more than 0.03 g DM per cm^2
   - sufficient sieving time to effect a complete separation of particles. Preferably no less than 20 min.
   - copious washing of samples before and during sieving to minimize matting of particles

b. It is possible to obtain representative samples of the rumen digesta, in terms of DM concentration and particle size distribution, with a simple instrument through a fistula.

c. Very few particles in the faeces are retained on sieves of aperture size larger than 1.18 mm

d. Ammonia-treatment of the straw and/or high levels of supplementation with lucerne pellets seem to have little or no effect on the rate of size reduction of large straw particles in the rumen.

e. Lucerne supplementation and, to a lesser extent, ammonia-treatment increase total intake, which is associated with increasing rates of escape of small straw particles from the rumen

f. Three sections of the digestive tract controlling the rate of passage of digesta are apparent from the excretion curves of chromium after the administration of Cr-mordanted straws. More critical research is needed to identify what these sections are in ruminants fed low- to medium-quality roughages.

CHAPTER 6

SUPPLEMENTATION OF BARLEY STRAWS WITH LUCERNE AND SUGAR BEET PULP IN DIETS FOR SHEEP

6.1. INTRODUCTION

In the experiments described in Chapter 4, the ME intake by sheep consuming barley straws was increased through supplementation with lucerne pellets. Generally, the diets containing lucerne at the highest level (480 g DM per kg total dietary DM) were adequate for maintenance only. For higher levels of production, straw-lucerne diets should be further supplemented with feeds high in energy. Ideally, these feeds should maintain, or preferably increase, the intake and digestibility of the basal diet and also increase animal performance. Feeds rich in easily digestible fibre such as green forages and sugar beet pulp have been shown to meet these requirements (Preston and Leng, 1984; Silva and Ørskov, 1985).

The beneficial effects of ammonia-treatment of straws on their feeding value for the ruminant are widely recognized (*e.g.* Greenhalgh, 1984; Nicholson, 1984; Sundstøl and Coxworth, 1984). However, these effects are strongly influenced by the nature and level of other components of the ration. Levels of starchy feeds above 0.3-0.4 of the total dietary DM decrease the rumen degradation (*in sacco*) and the ME value of ammonia-treated straws (Fahmy *et al*, 1984; Horton, 1978; Horton and Steacy, 1979). Maximum benefits from chemical treatment of straws may be obtained if they are supplemented with feeds that maintain or increase their feeding value.

The objective of the present work was to study the effect of supplementing untreated and ammonia-treated straw with lucerne and sugar beet pulp on the *in sacco* degradation, the *in vivo* digestibility and intake of the dietary

components. Some fermentation parameters in the rumen of sheep fed the experimental diets were also studied.

6.2. MATERIALS AND METHODS

6.2.1. Supplementation of untreated straw with lucerne and sugar beet pulp

6.2.1.1. Experiment 7: *In sacco* degradation of straw and rumen fermentation

a. Animals and management

Twelve Suffolk-cross wethers (59.7 ±1.8 kg LW) with permanent rumen cannulae (40 mm diameter) were used. They were harnessed for separate collection of urine and faeces and kept in individual crates under continuous illumination.

b. Diets and feeding

Untreated barley straw (US) (*c.v.* Golden Promise) was coarsely shredded in a small-bale tub grinder. The straw was offered *ad libitum* and supplemented with pellets at the ratio of 320 g per kg total diet DM basis, containing the following proportions of dried lucerne (L) and unmolassed sugar beet pulp (BP) (g per kg, DM basis): 1000L: 0BP (Diet US-0BP); 667L: 333BP (Diet US-33BP); 333L: 667BP (Diet US-67BP) and 0L: 1000BP (Diet US-100BP). A level of supplementation of 320 g per kg diet was adopted with the aim of producing diets which could sustain maintenance and moderate levels of production in different classes of ruminant livestock. The composition of the straw and the lucerne used is given in Table 4.1. The BP used had the following composition (g per kg DM): total ash (75); total N (15); NDF (469) and MADF (251) and an *in vitro* OMD of 0.876. Sufficient pellets containing different proportions of L and BP were prepared for all the experiments described in this chapter. The feeding procedures were similar to those described for Experiment 2 (Chapter

4). The daily allowance of feed was offered in equal amounts at 9:00 and 17:00 h. The straw was given after the pellets were completely consumed. At each feeding, a solution containing the required amount of urea to supply the estimated RDP requirements of the rumen microbes (ARC, 1984), was thoroughly mixed with the straw (300 ml per kg straw). These requirements were calculated using the values for CP, ME and CP degradability in lucerne determined in previous trials. Similarly, the determined CP and ME of the straw were used. CP degradabilities of 0.50 and 0.64 were assumed for the straw (Section 2.6.3.2) and the BP (ARC, 1984), respectively. A premix of minerals and vitamins containing sodium sulphate (Table 3.1 A) to attain a RDN: S ratio of 14: 1 (ARC, 1980) was also mixed with the straw. Water was freely available. Other feeding procedures were as described in previous chapters.

The experiment consisted of a 14-day adjustment period followed by two periods when five dacron bags containing straw were incubated in the rumen. Rest periods of 48 h were allowed between these incubation periods. Samples of rumen fluid were withdrawn at 0, 2, 4 and 7 h post-morning feeding on the day before and the day after the incubation of bags. Rumen pH, NH_3-N and VFA were determined in the samples.

c. *In sacco* degradation of straw

The degradation of straw in the rumen was measured using the dacron bag technique as outlined for Experiment 4 (Chapter 4); except that only the degradation of straw DM was determined. Straw was incubated in the rumen of the sheep for 5, 11, 24, 48 and 72 h.

6.2.1.2. Experiment 8: Intake and *in vivo* digestibility of diets containing untreated straw, lucerne and sugar beet pulp

a. Animals and management

Sixteen Suffolk-cross wethers of 32.8 ±0.6 kg LW were used. They were harnessed for collection of faeces and kept in individual crates designed for direct collection of urine. Continuous artificial illumination was maintained throughout the trial.

b. Diets and feeding

The diets and feeding management were as for Experiment 7, except that the allowance of pellets was adjusted every two days. The experimental period consisted of 10 days when only urea-supplemented straw was fed to the sheep, 14 days for adjustment to the experimental diets and 13 days for recording of intake and collection of faeces and urine.

c. *In vivo* digestibility of the diets

The *in vivo* digestibilities of DM, OM, CP, NDF, MADF and GE in the diets were determined by standard procedures (Schneider and Flatt, 1975). The ME concentration of the diets was calculated using predicted values for methane (Blaxter and Clapperton, 1965). Urine and faeces were collected daily for 10 days and bulked. Faeces were stored frozen while urine was stored at room temperature. The urine pH was adjusted daily to 3.5 by addition of 0.25 (v/v) sulphuric acid. The urine-collecting funnels were rinsed twice daily with 0.02 (v/v) sulphuric acid. Samples of the feeds were collected daily throughout the trial and bulked. Subsamples of the feeds, urine and faeces were taken for chemical analyses.

6.2.2. Supplementation of ammonia-treated straw with lucerne and sugar beet pulp

6.2.2.1. Experiment 9: *In sacco* degradation of straw and rumen fermentation

a. Animals and management

Twelve Suffolk-cross wethers of 62.2 ±1.6 kg LW were used. They were rumen-fistulated, harnessed and housed as in Experiment 7.

b. Diets and feeding

Ammonia-treated barley straw (TS) was used. This was from the same batch of straw used in Experiments 4 to 6 (See Table 4.1). The straw was fed *ad libitum* and supplemented with pellets at a ratio of 480 g per kg of total diet (DM basis) containing the following proportions of dried lucerne (L) and unmolassed sugar beet pulp (BP) (g per kg, DM basis): 1000L: 0BP (Diet TS-0BP); 667L: 333BP (Diet TS-33BP); 333L: 667BP (Diet TS-67BP) and 0L: 1000BP (Diet TS-100BP). A level of supplementation of 480 g per kg of total diet was adopted with the aim of developing diets comparable to those based on straw and grain, usually barley, which have been shown to promote high levels of production (Owen, 1984; Strickland, 1984). Other feeding procedures were as in Experiment 7, except that urea was added to diets TS-67BP and TS-100BP only.

Experimental periods for adjustment, incubation of dacron bags and sampling of rumen fluid were as in Experiment 7.

c. *In sacco* degradation of straw

The rumen degradation of treated-straw DM was determined using the dacron bag technique as outlined for Experiment 7.

6.2.2.2. Experiment 10: Intake and *in vivo* digestibility of diets containing treated straw, lucerne and sugar beet pulp

a. Animals and management

Sixteen Suffolk-cross wethers of 35.5 ±0.5 kg LW were used. They were harnessed, crated and housed as in Experiment 8.

b. Diets and feeding

The diets and feeding management were as in Experiment 9, except that the allowance of pellets was adjusted every two days. The experiment consisted of 10 days when the sheep were fed treated-straw alone, 14 days for adjustment to the experimental diets and 13 days for recording of intake and collection of faeces and urine.

c. *In vivo* digestibility of the diets

The same procedures described for Experiment 8 were used.

6.2.3. Experiment 11: *In vivo* digestibility of pellets containing lucerne and sugar beet pulp

6.2.3.1. Animals and management

Sixteen Suffolk-cross wethers of 41.5 ±0.7 kg LW were used. They were harnessed, crated and housed as described for previous *in vivo* digestibility trials.

6.2.3.2. Diets and feeding

Pellets containing the following proportions of dried lucerne (L) and unmolassed sugar beet pulp (BP) (g per kg, DM basis) were fed to supply the estimated ME requirements for maintenance of the sheep (ARC, 1980): 1000L : 0BP (Diet 0BP); 667L: 333BP (Diet 33BP); 333L: 667BP (Diet 67BP) and 0L:

1000BP (Diet 100BP). The experiment consisted of 14 days of adjustment followed by 13 days for collection of faeces and urine. Due to a shortage of feed the digestibility determination for diet OBP (lucerne pellets) could not be completed.

6.2.3.3. *In vivo* digestibility of the diets

The same procedures described for previous digestibility trials were used.

6.2.4. CHEMICAL ANALYSES

In all experiments, the analysis of feed, faeces and urine were done as detailed in Chapter 3, except that total N in ammonia-treated straw was determined in fresh hand-chopped samples using the macro Kjeldahl procedure

6.2.5. STATISTICAL ANALYSES

The dietary treatments in the *in sacco* degradation studies with untreated (Experiment 7) and treated (Experiment 9) straw were arranged in a completely randomized block design with three sheep per diet and blocks according to LW at the beginning of the trial. The *in sacco* degradation data for straw DM were analyzed by analysis of variance (ANOVA) for a nested type design with three strata: diets as whole units; incubation periods as sub-units and incubation times as sub-sub-units (Snedecor and Cohran, 1980; Steel and Torry, 1960). The diet effect was partitioned into its linear and quadratic components. Following the recommendations of Steel and Torry (1960) for nested designs in time, the pooled residual sum of squares for each stratum was initially partitioned into the respective block interactions. The effects of diet, incubation period and incubation time were then tested against the respective block interaction in the residual mean square or against the pooled residual mean square for the stratum . If these tests gave similar results the pooled residual mean square remained in the model of the design. A similar statistical

anlysis was performed for rumen pH and NH_3-N except that diets were the whole units, sampling days the sub-units and times the sub-sub-units. The determination of VFA was done on samples obtained by mixing equal volumes of rumen fluid from each of the sampling days. The corresponding statistical analysis was done as for a nested design with two strata: diets as whole units and times as sub-units.

The significance of the diet effect (level of lucerne and sugar beet pulp) on the intake and *in vivo* digestibility of diets based of untreated (Experiment 8) or treated (Experiment 10) straw was tested by ANOVA for a completely randomized block design with four replicates per treatment. This effect was partitioned into its linear and quadratic components. The sheep were blocked according to their straw intake during the first ten days of the experiments, when the straws were fed alone. Linear regressions of sugar beet pulp OMI on straw OMI, total OMI or total DOMI were calculated for Experiment 8 and Experiment 10. Digestibility data for the pellets containing different proportions of lucerne and sugar beet pulp were analyzed by ANOVA for a completely randomized design with four replicates per treatment.

6.3. RESULTS

6.3.1. Supplementation of untreated straw with lucerne and sugar beet pulp

6.3.1.1. Experiment 7: *In sacco* degradation of straw and rumen fermentation

a. Intake and *in sacco* degradation of straw

The straw OMI and total OMI were similar among diets ($P<0.05$) (Table 6.1). The coefficients of the linear effect of diet on both straw and total OMI were very close to zero and not significant ($P<0.05$) (Table 6.1A).

TABLE 6.1 Intake by rumen-fistulated sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp (Experiment 7).

Daily OMI g per kg $LW^{0.75}$	Diet description				
	US-0BP	US-33BP	US-67BP	US-100BP	s.e. means
Straw	21.5	16.8	21.1	19.4	2.78
Supplement[1]	9.5	8.0	9.5	8.8	-
Total	31.0	24.8	30.6	28.2	3.80

1. Pellets containing various proportions of lucerne and sugar beet pulp, fed at 320 g DM/kg total dietary DM.

At all incubation times, there was no significant effect of the composition of supplement on the *in sacco* degradation of straw DM ($P<0.05$) (Table 6.2). However, the *in sacco* degradation of straw at 24, 48 and 72 h was slightly higher for diet US-100BP than for diet US-OBP. For all diets and most incubation times, the *in sacco* degradation of straw was lower during incubation period 1 than during period 2 ($P<0.01$) (Tables 6.2 and 6.2A). In this experiment and Experiment 9, where the *in sacco* degradation of straw DM and rumen fermentation parameters were analyzed by ANOVA for nested designs, most block interactions were non-significant. Therefore, these interactions were removed from the statistical models and are not shown in the ANOVAs detailed in the tables of the Appendix.

b. Rumen fermentation parameters

The pH and NH_3-N concentration in the rumen fluid were very similar in sheep receiving untreated straw and pellets with different proportions of sugar beet pulp and lucerne ($P<0.05$) (Figure 6.1 and Table 6.3A). pH decreased rapidly from about 6.8 at 0 h post-feeding to about 6.4 at 4 h post-feeding and thereafter remained fairly constant up to 7 h post-feeding. The concentration of NH_3-N in the rumen fluid of the sheep increased significantly between 0 and 2 h post-feeding and then declined at 4 and 7 h post-feeding to levels similar to those at 0 h ($P<0.001$).

At all sampling times, the total concentration of VFA in the rumen was not affected by the composition of the supplement. Generally, the s.e. of the means at each sampling time were large (Figure 6.2 and Table 6.4A). The total concentration of VFA in the rumen was highest between 2 and 4 h post-feeding for all diets. At 4 h post-feeding the differences among diets were large but did not reach statistical significance ($P<0.05$). The molar

TABLE 6.2 *In sacco* degradation (g/g) of straw DM in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp.

Period	Incubation time, h	Diet description[1]					
		US-0BP	US-33BP	US-67BP	US-100BP	**Mean**	s.e. means
1	5	0.075	0.069	0.080	0.071	**0.074**	0.0059
	11	0.193	0.198	0.160	0.189	**0.185**	0.0126
	24	0.317	0.325	0.253	0.324	**0.305**	0.0198
	48	0.428	0.442	0.415	0.443	**0.432**	0.0129
	72	0.477	0.487	0.494	0.505	**0.491**	0.0086
	Mean	**0.298**	**0.304**	**0.280**	**0.306**	**0.297**[2]	
2	5	0.105	0.098	0.107	0.105	**0.104**	0.0047
	11	0.185	0.208	0.191	0.180	**0.191**	0.0138
	24	0.334	0.356	0.333	0.366	**0.347**	0.0225
	48	0.466	0.477	0.442	0.471	**0.464**	0.0181
	72	0.516	0.531	0.484	0.525	**0.514**	0.0117
	Mean	**0.321**	**0.334**	**0.311**	**0.329**	**0.324**[2]	
	Overall mean	**0.310**	**0.319**	**0.296**	**0.318**		

1. Straw and pelleted supplements containing various proportions of lucerne and sugar beet pulp, fed at 320 g DM/kg total dietary DM.
2. s.e. diff. of overall period means = 0.006.

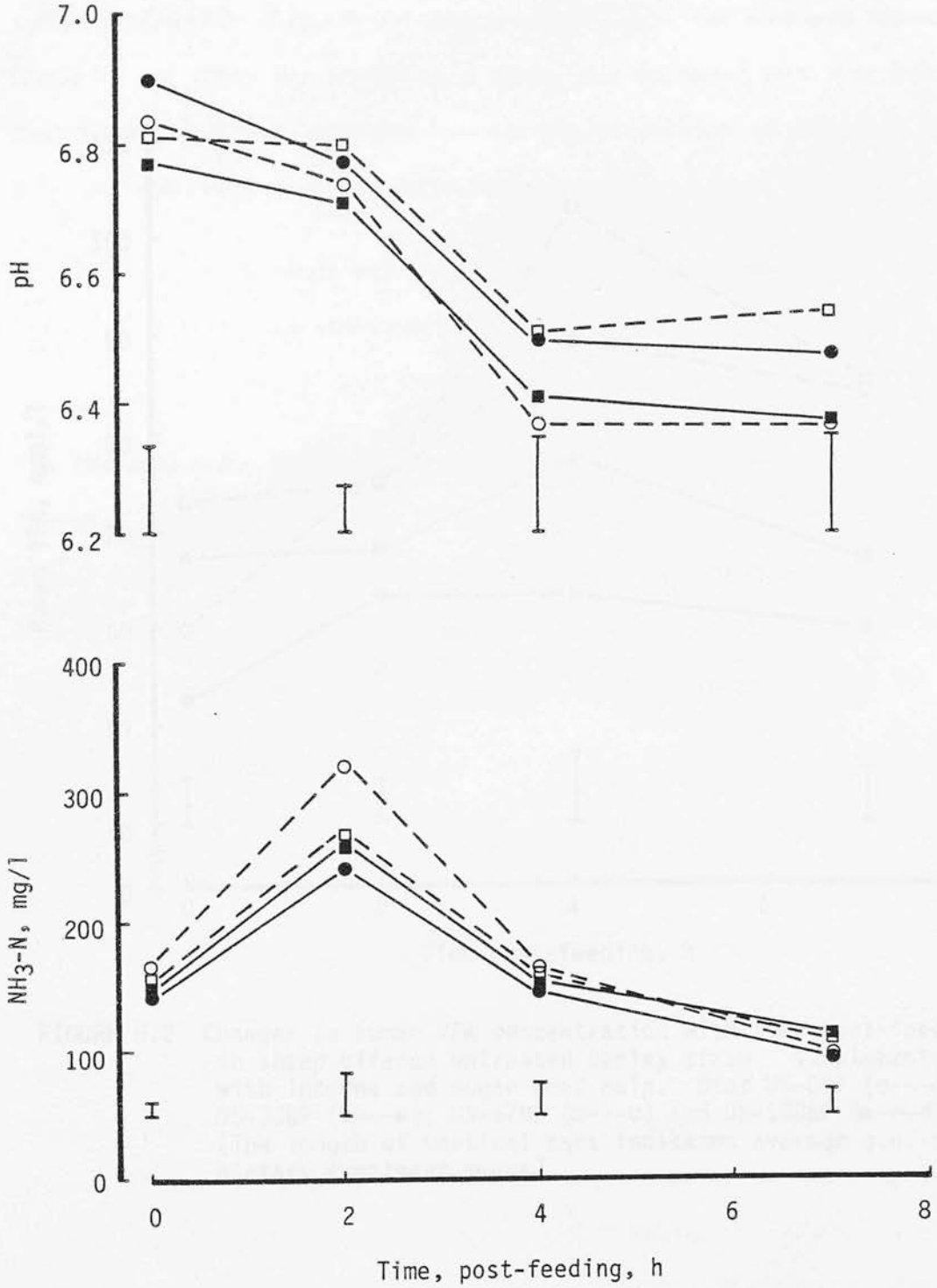

FIGURE 6.1 Changes in rumen pH and NH_3-N levels with time post-feeding in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp. Diet US-0BP (○--○); US-33BP (●—●); US-67BP (□--□) and US-100BP (■—■). (The length of vertical bars indicates s.e. means).

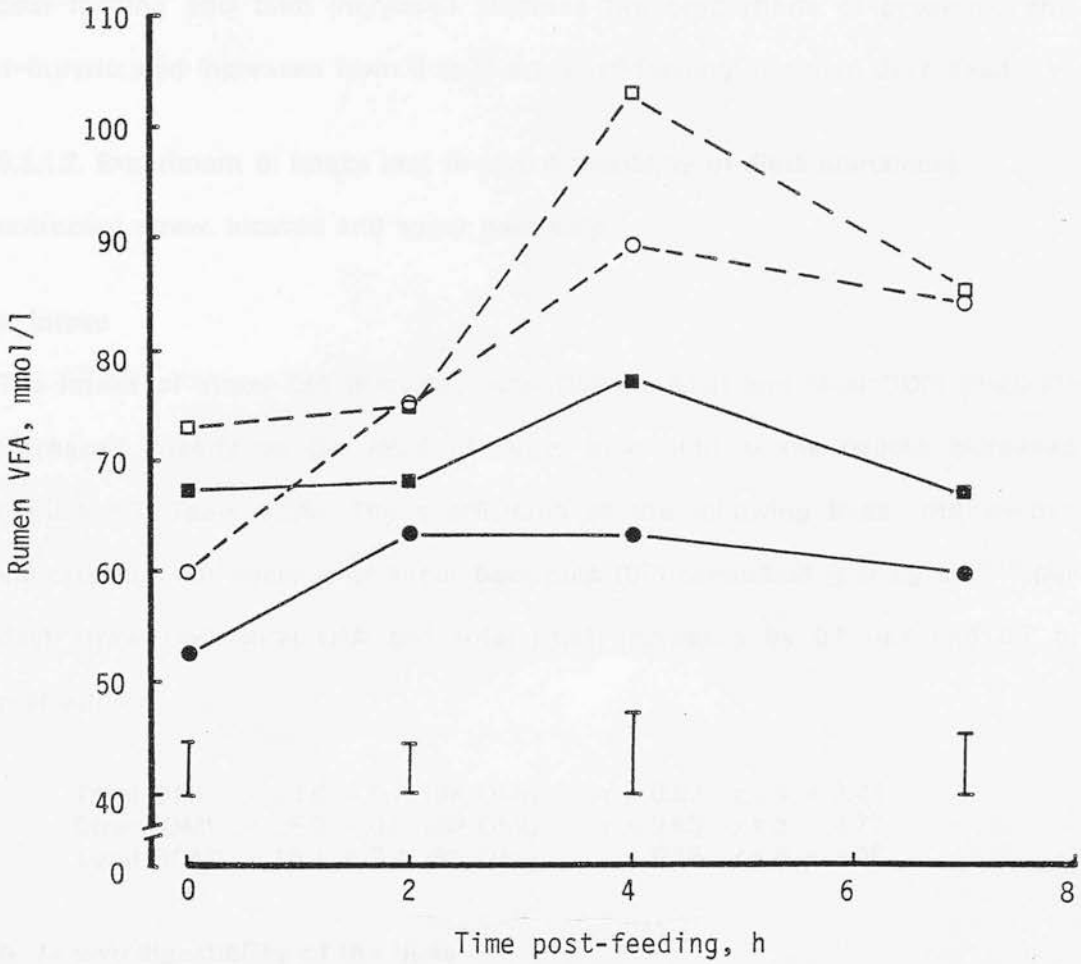

FIGURE 6.2 Changes in rumen VFA concentration with time post-feeding in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp. Diet US-0BP (o---o); US-33BP (●——●); US-67BP (□---□) and US-100BP (■——■). (The length of vertical bars indicates average s.e. of dietary treatment means).

proportions of acetic, propionic and n-butyric acid in the rumen fluid were similar among diets ($P<0.05$) and changed significantly with time post-feeding (Table 6.3 and 6.4A). The proportion of acetic acid decreased from 0 to 2-4 h post-feeding and then increased whereas the proportions of propionic and n-butyric acid increased from 0 to 2-4 h post-feeding and then decreased.

6.3.1.2. Experiment 8: Intake and *in vivo* digestibility of diets containing untreated straw, lucerne and sugar beet pulp

a. Intake

The intake of straw OM ($P<0.05$), total OM ($P<0.05$) and total DOM ($P<0.01$) increased linearly as the level of sugar beet pulp in the pellets increased (Figure 6.3, Table 6.5A). The coefficients of the following linear regressions indicate that for every g of sugar beet pulp (BP) consumed (per kg $LW^{0.75}$ per day) straw OMI, total OMI and total DOMI increased by 0.5, 0.7 and 0.7 g, respectively:

Total OMI	= 36.0 + 0.7 (BP OMI)	r = 0.62	r.s.d. = 3.41
Straw OMI	= 25.3 + 0.5 (BP OMI)	r = 0.63	r.s.d. = 4.77
Total DOMI	= 18.4 + 0.7 (BP OMI)	r = 0.76	r.s.d. = 3.05

b. *In vivo* digestibility of the diets

The diets, as offered to the sheep, were similar in their chemical analyses (Table 6.4). The *in vivo* digestibilities of DM, OM, NDF, MADF, and GE in the diets increased linearly ($P<0.01$) as the level of sugar beet pulp increased (Figure 6.4 and Table 6.5A). The *in vivo* digestibilities of all these fractions were lower for diet US-100BP than for diet US-67BP, but the differences were not significant ($P<0.05$). The deviations from linearity of the effect of diet on the *in vivo* digestibility of DM, OM, NDF, MADF and GE were non-significant ($P<0.05$) (Table 6.5A).

TABLE 6.3 Changes in molar proportions (mmol/mmol) of acetic, propionic and n-butyric acid in the rumen with time post-feeding, in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp[1].

Acid		Time post-feeding, h			
		0	2	4	7
Acetic	Mean[2]	0.731	0.725	0.736	0.751
	s.e.	0.0042	0.0049	0.0050	0.0041
Propionic	Mean	0.176	0.181	0.178	0.171
	s.e.	0.0034	0.0041	0.0040	0.0027
n-Butyric	Mean	0.062	0.066	0.065	0.060
	s.e.	0.0030	0.0024	0.0019	0.0021

1. Pellets fed at 320 g DM/kg total dietary DM.
2. Overall mean for sheep on all treatments.

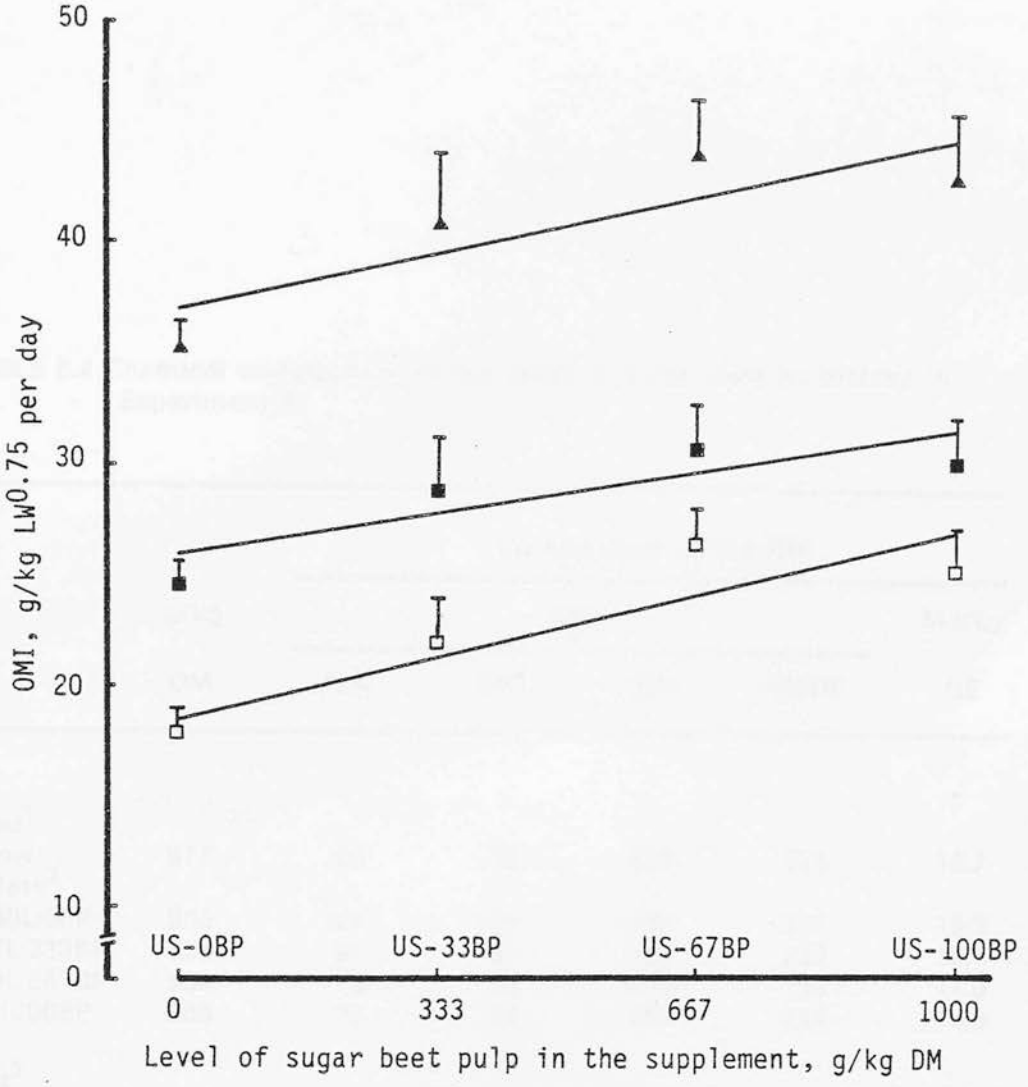

FIGURE 6.3 Relationships between the level of sugar beet pulp in the supplement and: total OMI (▲—▲); straw OMI (■—■) and total DOMI (□—□) in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp. (320 g pellets/kg total diet, DM basis). (The linear effects were significant for straw OMI, total OMI and total DOMI, see Table 6.5A; each symbol indicates the mean of 4 observations and the length of vertical bars the s.e. mean).

TABLE 6.4 Chemical composition of the feeds and the diets as offered in Experiment 8.

	g/kg	Composition of the DM				
		g/kg				MJ/kg
	DM	Ash	CP[4]	NDF	MADF	GE
Feed[1]						
Straw	877	50	32	838	513	18.7
Pellets[2]						
1000L:0BP	855	104	160	486	301	18.3
667L:333BP	855	93	139	492	283	17.7
333L:667BP	852	88	122	479	253	17.0
0L:1000BP	859	76	92	459	244	16.8
Diet[3]						
US-0BP	870	68	89	725	445	18.6
US-33BP	870	64	90	730	439	18.4
US-67BP	869	60	94	725	430	18.2
US-100BP	871	58	92	720	427	18.1

1. Determined composition.
2. Lucerne (L, g/kg): sugar beet pulp (BP, g/kg), DM basis.
3. Calculated values using the chemical composition of the feeds.
4. Including urea-N added to the diets.

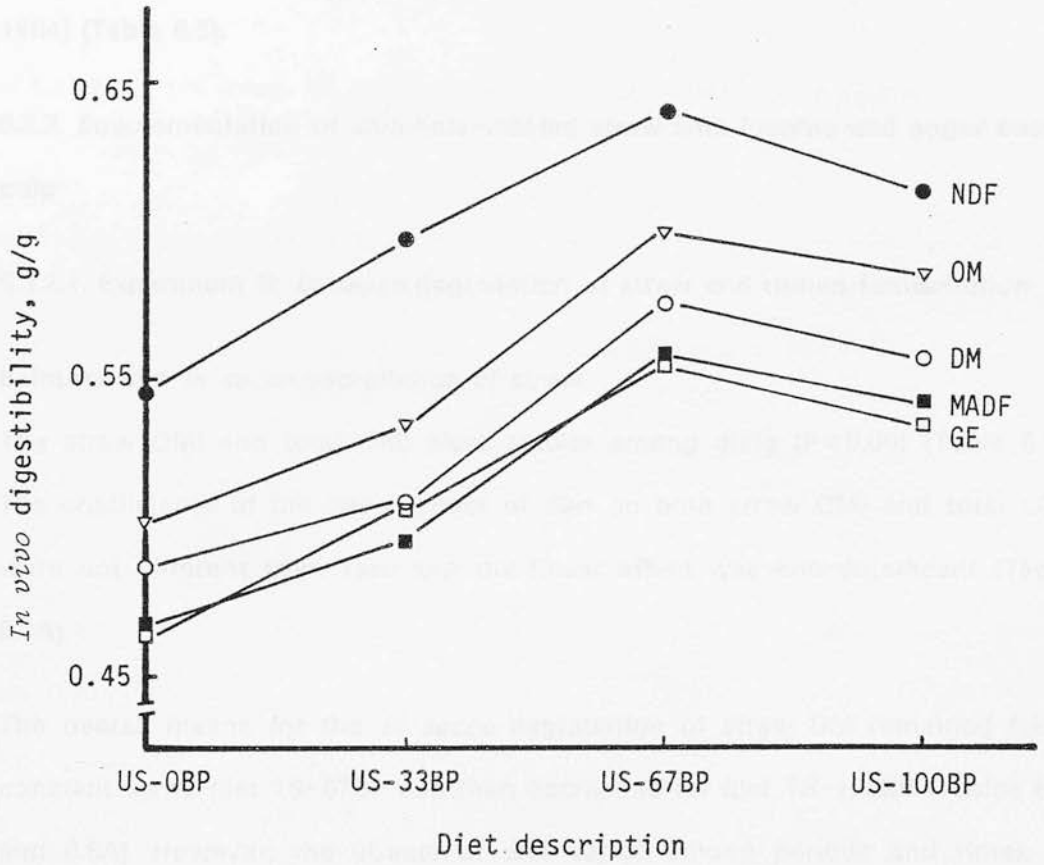

FIGURE 6.4 *In vivo* digestibility of the diets in sheep offered untreated barley straw supplemented with pellets (320 g DM/kg dietary DM) containing lucerne and sugar beet pulp. (s.e. means: DM = 0.018; OM = 0.018; GE = 0.021; NDF = 0.018; MADF = 0.020).

The ME concentration of the diets and the daily ME intake increased linearly as the level of sugar beet pulp increased (Table 6.5) with no deviations from linearity (Table 6.5A). As the level of sugar beet pulp increased, N intake, N excreted in urine, N-balance and CP digestibility increased linearly while N excreted in faeces remained constant (Table 6.5). The RDP/ME ratios for all diets were above the estimated requirements of the rumen microbes (ARC, 1984) (Table 6.5).

6.3.2. Supplementation of ammonia-treated straw with lucerne and sugar beet pulp

6.3.2.1. Experiment 9: *In sacco* degradation of straw and rumen fermentation

a. Intake and *in sacco* degradation of straw

The straw OMI and total OMI were similar among diets ($P<0.05$) (Table 6.6). The coefficients of the linear effect of diet on both straw OMI and total OMI were not different from zero and the linear effect was non-significant (Table 6.1A).

The overall means for the *in sacco* degradation of straw DM remained fairly constant up to diet TS-67BP and then decreased for diet TS-100BP (Tables 6.7 and 6.6A). However, the effects of diet varied among periods and times of incubation, as indicated by the significant effects ($P<0.001$) of period, incubation time and incubation time x diet (Table 6.6A). The *in sacco* degradation of straw DM was higher ($P<0.01$) during incubation period 2 than during period 1. The decrease in the *in sacco* degradation of straw DM between the extreme diets TS-OBP and TS-100BP was largest at 24 h of incubation and smallest at 5 and 72 h, with intermediate values at 11 and 48 h.

TABLE 6.5 ME of the diets, ME intake, N-balance data and CP digestibility in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp.

	Diet description[1]				s.e. means	Linear effect	
	US-0BP	US-33BP	US-67BP	US-100BP		Coeffic. (x 10^5)	Signif.
ME							
In the diet (MJ/kg DM)	6.8	7.5	8.1	8.0	0.38	121	*
Daily intake (MJ per sheep)	3.5	4.6	5.1	5.1	0.35	157	**
Nitrogen (g per sheep per day)							
Intake	7.2	8.5	9.3	9.4	0.44	217	**
Faeces	1.4	1.6	1.4	1.4	0.11	-	NS
Urine	4.2	4.8	5.5	5.2	0.26	106	*
Balance	1.6	2.1	2.4	2.8	0.33	120	*
CP digestibility (g/g)	0.803	0.811	0.853	0.850	0.0106	6	**
RDP/ME (g/MJ)[2]	9.0	8.1	7.9	7.9	-	-	-

1. Straw and pelleted supplement containing various proportions of lucerne and sugar beet pulp, fed at 320 g DM/kg total dietary DM.
2. Assuming rumen degradabilitites of CP in straw, lucerne and sugar beet pulp of 0.50, 0.75 and 0.64, respectively.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS: not significant

TABLE 6.6 Intake by rumen-fistulated sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp (Experiment 9).

Daily OMI g per kg $LW^{0.75}$	Diet description				
	TS-0BP	TS-33BP	TS-67BP	TS-100BP	s.e. means
Straw	32.8	31.1	31.3	29.7	2.73
Supplement[1]	28.9	28.4	28.4	26.5	-
Total	61.7	59.5	59.7	56.2	5.23

1. Pellets containing various proportions of lucerne and sugar beet pulp, fed at 480 g DM/kg total dietary DM.

TABLE 6.7 *In sacco* degradation (g/g) of straw DM in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

Period	Incubation time, h	Diet description[1]					
		TS-0BP	TS-33BP	TS-67BP	TS-100BP	**Mean**	s.e. means
1	5	0.178	0.157	0.197	0.169	**0.175**	0.0096
	11	0.308	0.286	0.297	0.255	**0.287**	0.0265
	24	0.480	0.477	0.523	0.344	**0.456**	0.0190
	48	0.606	0.599	0.659	0.542	**0.601**	0.0154
	72	0.675	0.689	0.690	0.654	**0.677**	0.0202
	Mean	**0.449**	**0.441**	**0.473**	**0.393**	**0.439**	
2	5	0.181	0.195	0.181	0.169	**0.182**	0.0086
	11	0.316	0.307	0.317	0.298	**0.310**	0.0196
	24	0.532	0.500	0.528	0.425	**0.496**	0.0202
	48	0.661	0.659	0.658	0.580	**0.640**	0.0149
	72	0.696	0.719	0.707	0.666	**0.697**	0.0210
	Mean	**0.477**	**0.476**	**0.478**	**0.428**	**0.465**	
	Overall mean	**0.463**	**0.459**	**0.476**	**0.410**		

1. Straw and supplements containing various proportions of lucerne and sugar beet pulp, fed at 480 g DM/kg total dietary DM.
   s.e. diff. of means:
   -between diets within incubation times within period = 0.025
   -between diets across incubation times within period = 0.018
   -between periods = 0.005

b. Rumen fermentation parameters

At all times post-feeding, the pH of the rumen fluid of sheep receiving diet TS-100BP was lower than corresponding values for the other three diets, although the overall effect of diet was non-significant (Figure 6.5, Table 6.7A). Rumen pH decreased sharply between 0 and 4 h post-feeding and then tended to stabilize for all diets.

The concentration of NH_3-N in the rumen increased between 0 and 2 h post-feeding and then decreased steadily until 7 h post-feeding fo all diets (Figure 6.5). Generally, rumen NH_3-N decreased linearly as the level of sugar beet pulp increased; the overall means from all post-feeding times and sampling periods were 227, 164, 179 and 172 mg NH_3-N per l of rumen fluid for diets TS-0BP, TS-33BP, TS-67BP and TS-100BP, respectively. This linear effect was not consistent at all post-feeding times, but the treatment means varied within relatively narrow ranges in most cases.

The total concentrations of VFA in the rumen of sheep receiving diets TS-OBP and TS-33BP reached a peak at 2 h post-feeding and then declined (Figure 6.6). This peak was reached longer after feeding (4 h) in diet TS-67BP. The total VFA in the rumen of sheep eating diet TS-100BP increased from 0 to 7 h post-feeding. The overall mean for total rumen VFA concentration for all post-feeding times increased linearly ($P<0.1$) with the level of sugar beet pulp in the pellets; from 93.6 mmol per l for diet TS-OBP to 118.8 mmol per l for diet TS-100BP.

The molar proportion of acetic acid in the rumen fluid decreased linearly whereas the molar proportion of n-butyric acid increased linearly as the level of sugar beet pulp in the diet increased ($P<0.05$) (Tables 6.8 and 6.8A). The molar proportion of propionic acid tended to increase from diet TS-OBP to

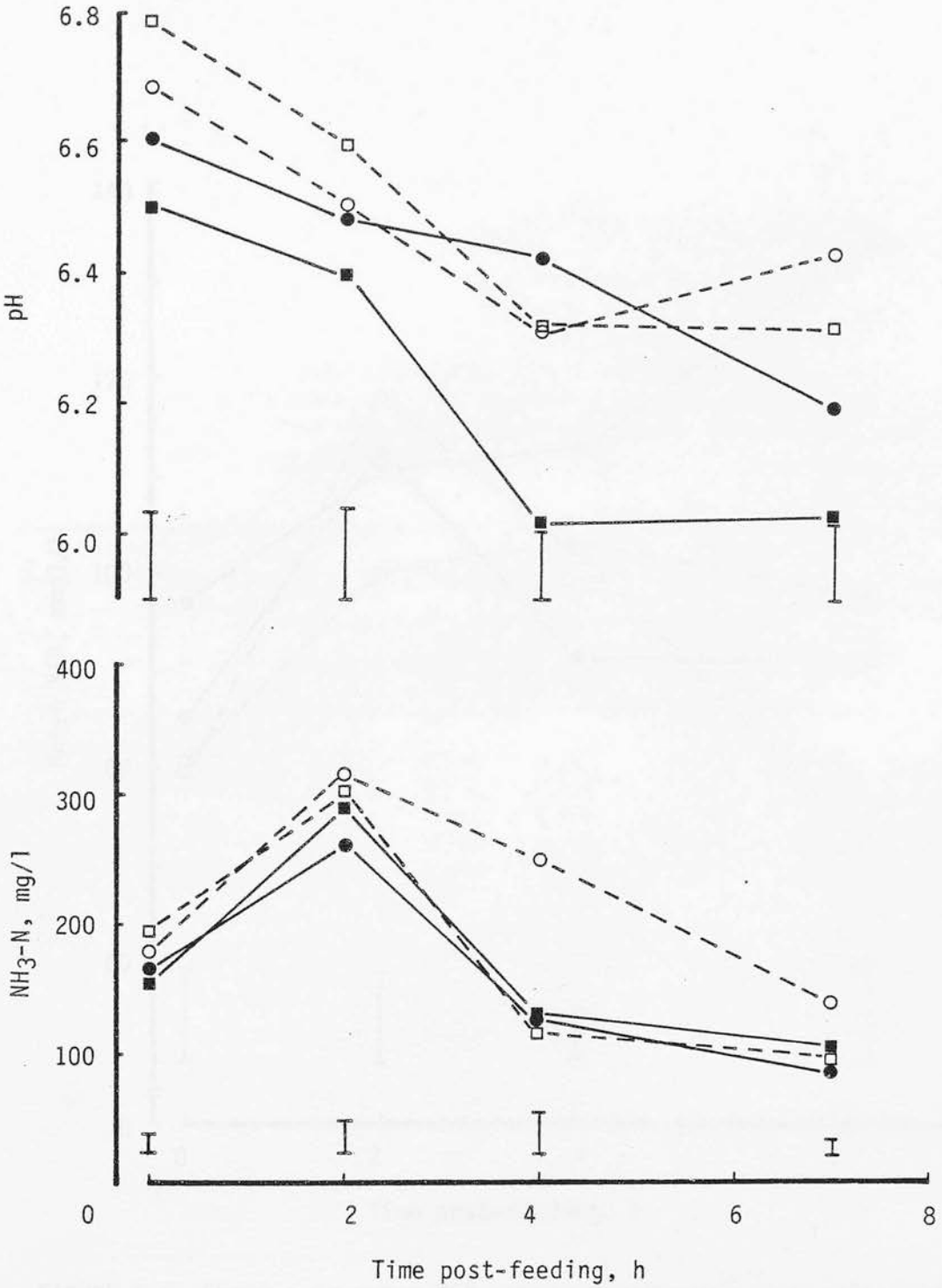

FIGURE 6.5 Changes in rumen pH and NH_3-N levels with time post-feeding in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp. Diet TS-0BP (o---o); TS-33BP (●——●); TS-67BP (□---□) and TS-100BP (■——■). (The length of vertical bars indicates s.e means).

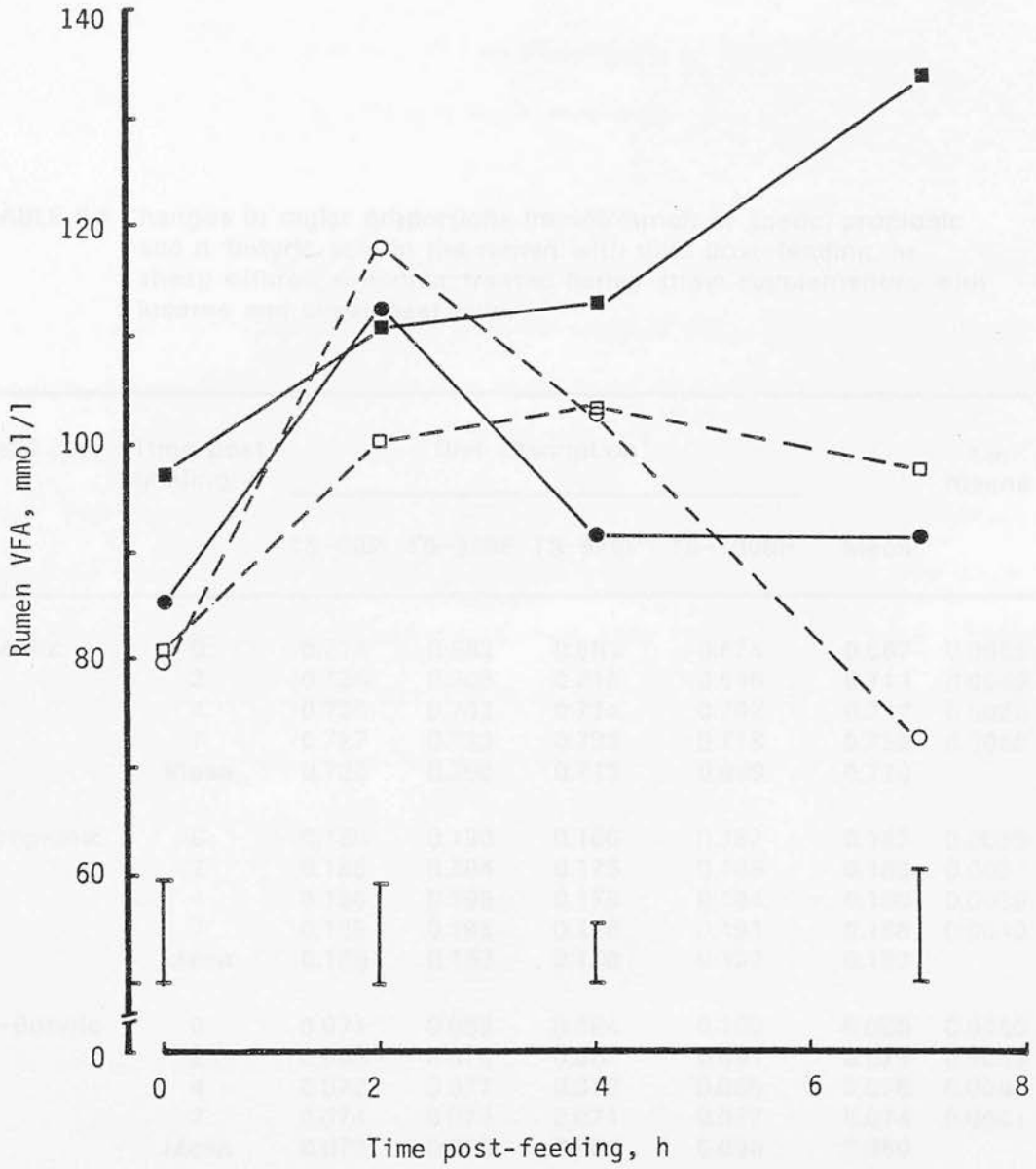

FIGURE 6.6 Changes in rumen VFA concentration with time post-feeding in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.
Diet TS-0BP (o---o); TS-33BP (●——●); TS-67BP (□---□) and TS-100BP (■——■). (The length of vertical bars indicate average s.e of treatment means).

TABLE 6.8 Changes in molar proportions (mmol/mmol) of acetic, propionic and n-butyric acid in the rumen with time post-feeding, in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

Acid	Time post feeding, h	Diet description[1]					s.e. means
		TS-0BP	TS-33BP	TS-67BP	TS-100BP	Mean	
Acetic	0	0.712	0.682	0.681	0.674	**0.687**	0.0058
	2	0.725	0.706	0.715	0.696	**0.711**	0.0069
	4	0.725	0.702	0.724	0.707	**0.717**	0.0060
	7	0.727	0.723	0.733	0.718	**0.725**	0.0060
	Mean	**0.722**	**0.706**	**0.713**	**0.699**	**0.710**	
Propionic	0	0.186	0.190	0.180	0.192	**0.187**	0.0035
	2	0.185	0.194	0.175	0.189	**0.186**	0.0037
	4	0.186	0.195	0.179	0.194	**0.188**	0.0039
	7	0.185	0.195	0.178	0.191	**0.188**	0.0040
	Mean	**0.185**	**0.193**	**0.178**	**0.192**	**0.187**	
n-Butyric	0	0.071	0.088	0.094	0.100	**0.088**	0.0050
	2	0.069	0.075	0.082	0.091	**0.079**	0.0055
	4	0.072	0.077	0.078	0.085	**0.078**	0.0042
	7	0.074	0.074	0.071	0.077	**0.074**	0.0041
	Mean	**0.072**	**0.079**	**0.081**	**0.088**	**0.080**	

1. Straw and pellets containing various proportions of sugar beet pulp and lucerne, fed at 480 g DM/kg total dietary DM.

s.e. of differences of means:

- -between diets within sampling times: acetic = 0.009; propionic = 0.005; n-butyric = 0.007
- -between sampling times within diet: acetic = 0.006; propionic = 0.003; n-butyric = 0.004
- -between diets across sampling times: acetic = 0.007; propionic = 0.004; n-butyric = 0.006

diet TS-100BP, but this effect was not significant. The molar proportion of acetic acid increased with time post-feeding whereas that of n-butyric acid decreased. The molar proportion of propionic acid did not change significantly with time after feeding.

6.3.2.2. Experiment 10: Intake and *in vivo* digestibility of diets containing ammonia-treated straw, lucerne and sugar beet pulp

a. Intake

The intakes of straw OM and total OM were similar among diets whereas the total intake of DOM increased linearly as the level of sugar beet pulp increased ($P<0.01$) (Figure 6.7, Table 6.9A). The coefficients of the linear regressions of total OMI on sugar beet pulp OMI and straw OMI on sugar beet pulp (BP) OMI were not significantly different from zero ($P<0.05$) and the regressions explained very little of the total variation. On the other hand, the linear regression of total DOMI on sugar beet pulp OMI explained about 0.6 of the total variation in total DOMI. The coefficient of this regression indicates that total DOMI increased by 0.3 g for every g of sugar beet pulp OM consumed. The regressions were:

Total OMI	= 57.1 + 0.051 (BP OMI)	r = 0.09	r.s.d. = 5.83
Straw OMI	= 31.6 + 0.002 (BP OMI)	r = 0.01	r.s.d. = 3.45
Total DOMI	= 31.9 + 0.306 (BP OMI)	r = 0.71	r.s.d. = 3.19

b. *In vivo* digestibility of the diets

The experimental diets, as offered to the sheep, had similar chemical analyses, except that diet TS-100BP was slightly lower in ash and MADF than diet TS-OBP (Table 6.9). There was a significant linear effect of diet composition ($P<0.001$) on the digestibility of DM, OM, GE, NDF, and MADF (Figure 6.8, Table 6.9A). However, in all cases, there were significant ($P<0.05$) deviations from

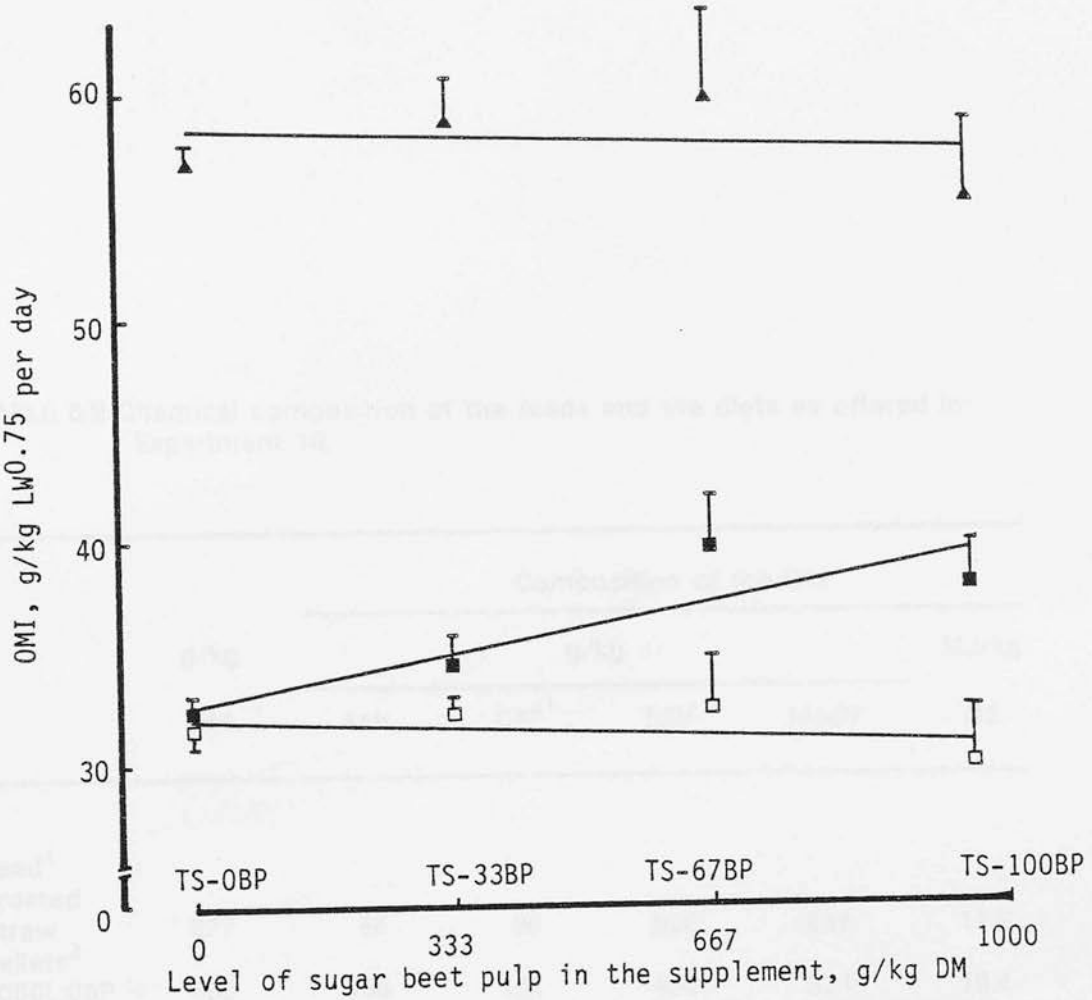

FIGURE 6.7 Relationships between the level of sugar beet pulp in the supplement and: total OMI (▲—▲); straw OMI (□—□) and total DOMI (■—■) in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp. (480 g pellets/kg total diet, DM basis). (The linear effect was significant for total DOMI only, see Table 6.9A; each symbol indicates the mean of 4 observations and the length of vertical bars the s.e. mean).

TABLE 6.9 Chemical composition of the feeds and the diets as offered in Experiment 10.

	g/kg	Composition of the DM				
		g/kg				MJ/kg
	DM	Ash	CP[4]	NDF	MADF	GE
Feed[1]						
Treated Straw Pellets[2]	877	56	96	802	522	17.9
1000L:0BP	853	106	161	490	321	18.4
667L:333BP	867	99	137	508	302	18.1
333L:667BP	852	85	116	493	284	17.4
0L:1000BP	837	74	97	480	258	17.1
Diet[3]						
TS-0BP	865	79	126	656	428	18.2
TS-33BP	872	76	115	664	419	18.0
TS-67BP	865	70	111	655	409	17.7
TS-100BP	858	64	119	650	397	17.5

1. Determined composition.
2. Lucerne (L, g/kg): sugar beet pulp (BP, g/kg), DM basis.
3. Calculated values using the chemical composition of the feeds.
4. Including urea-N added to the diets.

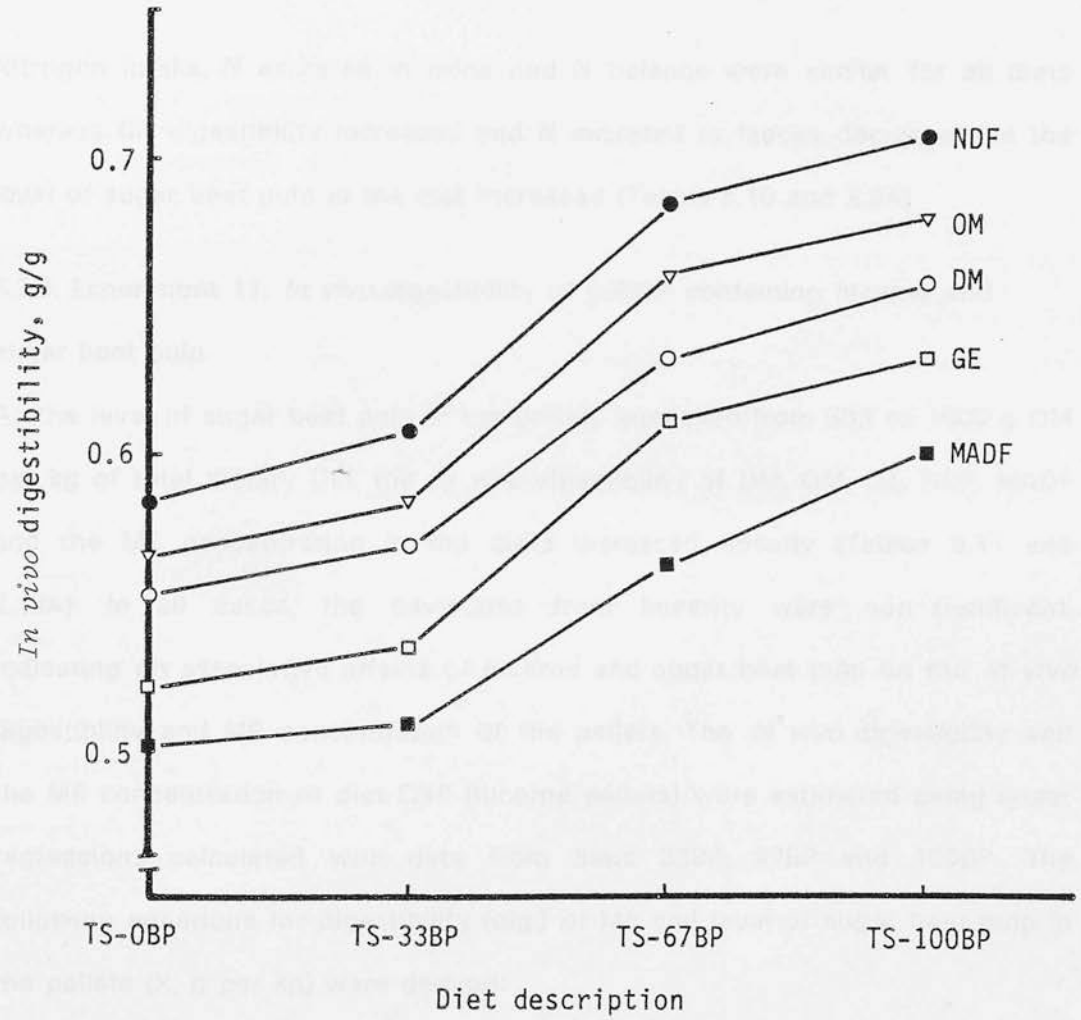

FIGURE 6.8 *In vivo* digestibility of the diets in sheep offered ammonia-treated barley straw supplemented with pellets (480g DM/kg dietary DM) containing lucerne and sugar beet pulp. (s.e. means: DM 0.007; OM = 0.008; GE = 0.009; NDF = 0.010; MADF = 0.011).

linearity, reflecting the larger increases between diets TS-OBP and TS-67BP compared to the increases between diets TS-67BP and TS-100BP (Figure 6.8). Similarly, the ME concentration of the diets and the daily ME intake increased linearly as the level of sugar beet pulp increased (Tables 6.10 and 6.9A), the deviation from linearity was significant only for the ME concentration of the diets (Tables 6.10 and 6.9A).

Nitrogen intake, N excreted in urine and N balance were similar for all diets whereas CP digestibility increased and N excreted in faeces decreased as the level of sugar beet pulp in the diet increased (Tables 6.10 and 6.9A)

6.3.3. Experiment 11: *In vivo* digestibility of pellets containing lucerne and sugar beet pulp

As the level of sugar beet pulp in the pellets increased from 333 to 1000 g DM per kg of total dietary DM, the *in vivo* digestibility of DM, OM, GE, NDF, MADF and the ME concentration in the diets increased linearly (Tables 6.11 and 6.10A). In all cases, the deviations from linearity were non-significant, indicating no associative effects of lucerne and sugar beet pulp on the *in vivo* digestibility and ME concentration of the pellets. The *in vivo* digestibility and the ME concentration of diet OBP (lucerne pellets) were estimated using linear regressions calculated with data from diets 33BP, 67BP and 100BP. The following equations for digestibility (dig.) or ME and level of sugar beet pulp in the pellets (X, g per kg) were derived:

		r	r.s.d
DM dig.	= 0.604 + 0.00020 X	0.96	0.018
OM dig.	= 0.611 + 0.00025 X	0.98	0.016
GE dig.	= 0.576 + 0.00025 X	0.97	0.018
NDF dig.	= 0.487 + 0.00039 X	0.98	0.024
MADF dig.	= 0.386 + 0.00039 X	0.91	0.037
ME	= 8.34 + 0.00362 X	0.96	0.296

TABLE 6.10 ME concentration of the diets, ME intake, N-balance data and CP digestibility in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

	Diet description[1]				s.e. means	Linear effect	
	TS-0BP	TS-33BP	TS-67BP	TS-100BP		Coeffic. ($\times 10^5$)	Signif.
ME							
In the diet (MJ/kg DM)	7.6	7.9	9.1	9.4	0.15	200	***
Daily intake (MJ per sheep)	6.8	7.3	8.4	8.3	0.35	171	**
Nitrogen (g per sheep per day)							
Intake	18.0	17.0	16.4	16.8	0.89	-	NS
Faeces	3.2	3.4	2.4	2.5	0.20	-91	**
Urine	5.2	5.0	4.3	5.0	0.77	-	NS
Balance	9.6	8.6	9.7	9.3	1.09	-	NS
CP digestibility (g/g)	0.823	0.800	0.850	0.851	0.0095	4	*
RDP/ME (g/MJ)[2]	11.6	9.8	8.1	8.4	-	-	-

1. Straws and pellets containing various proportions of sugar beet pulp and lucerne, fed at 480 g DM/kg total dietary DM.
2. Assuming rumen degradabilitites of lucerne CP, sugar beet pulp CP, original straw CP and CP added by ammoniation of 0.75, 0.64, 0.50 and 0.70, respectively.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$; NS: not significant

TABLE 6.11 Intake, *in vivo* digestibility and ME concentration of the diets and N-balance data in sheep fed pellets containing different proportions of lucerne and sugar beet pulp.

	Diet description[1]				s.e. means	Linear effect	
	0BP[2]	33BP	67BP	100BP		Coeffic. ($\times 10^5$)	Signif.
Daily OMI							
(g/kg $LW^{0.75}$)	-	36.3	34.3	27.2	1.56	-	-
Digestibility g/g							
DM	0.604	0.670	0.742	0.804	0.009	20.2	***
OM	0.611	0.691	0.785	0.857	0.008	25.0	***
Energy	0.576	0.658	0.753	0.827	0.009	25.4	***
NDF	0.487	0.619	0.751	0.883	0.012	39.6	***
MADF	0.386	0.520	0.638	0.781	0.019	39.1	***
CP	-	0.827	0.825	0.827	0.007	-	NS
ME							
In the diet							
(MJ/kg DM)	8.4	9.6	10.9	12.0	0.15	363	***
Daily intake							
(MJ per sheep)	-	6.6	6.8	5.5	0.33	-	NS
Nitrogen							
(g per sheep per day)							
Intake	-	15.0	11.5	7.2	0.40	-1170	***
Faeces	-	2.6	2.0	1.2	0.09	-204	***
Urine	-	8.4	5.7	3.2	0.20	-793	***
Balance	-	4.0	3.8	2.8	0.27	-	NS

1. Pellets containing various proportions of lucerne and sugar beet pulp.
2. Calculated values using linear regressions obtained with data from the other three dietary treatments; see text for explanation.

As the level of sugar beet pulp in the pellets increased, N intake and N excreted in faeces and urine decreased linearly. Nitrogen balance also decreased as the level of sugar beet pulp increased, but the linear effect was non-significant (Tables 6.11 and 6.10A).

6.4. DISCUSSION

6.4.1. Supplementation of untreated barley straw with lucerne and sugar beet pulp

6.4.1.1. *In sacco* degradation of straw and rumen fermentation

The objective of replacing lucerne by sugar beet pulp in supplements for untreated barley straw was to increase the total ME intake in two ways: a) directly, by the higher ME concentrations of the beet pulp and b) indirectly by an increased digestibility of the straw resulting from possible stimulating effects of sugar beet pulp on the cellulolytic activity of the rumen microbes (Silva and Ørskov, 1985). In the present work, there was some indication of an improved activity of fibre-degrading microbes in the rumen. The *in sacco* degradation of untreated straw at 24, 48 and 72 h of incubation was slightly higher for diet US-100BP than for diet US-OBP (Table 6.2). However, the differences were small and non-significant. Silva and Ørskov (1985) found that the *in sacco* degradation of straw in the rumen of sheep fed barley straw supplemented with 150 g of unmolassed sugar beet pulp per kg of total diet was higher than that in the rumen of sheep fed straw alone (0.503 *v.* 0.463 at 48 h). Two main reasons may explain the results obtained here. Firstly, the s.e. were larger, the differences between means were smaller and there were less replicates per treatment in the present trial than in the work of Silva and Ørskov (1985). Secondly, Silva and Ørskov (1985) compared the *in sacco* degradation of straw in the rumen of sheep fed straw alone with that in the

rumen of sheep fed supplemented straw. In Experiment 7, diet US-OBP (which contained 680 g straw and 320 g lucerne per kg total diet) was compared with diet US-100BP (which contained straw and 320 g sugar beet pulp per kg total diet). It was observed previously (Experiement 2, Chapter 4) that the *in sacco* degradation of straw in the rumen of sheep fed a diet corresponding to diet US-OBP tended to be higher than that in the rumen of sheep fed straw alone. Thus, if it had been compared in the same experiment, the *in sacco* degradation of straw in the rumen of sheep consuming diet US-100BP may have differed significantly from that in the rumen of sheep fed straw alone.

At the level of supplementation studied in Experiment 7, increasing the amount of sugar beet pulp did not change the parameters of rumen fermentation. As expected, rumen pH decreased with time post-feeding, but it was always above the critical value below which the activity of fibre-degrading bacteria is impaired (Mould *et al*, 1983). Despite increasing levels of urea from diet US-OBP to diet US-100BP, the concentrations of NH_3-N in the rumen were similar among diets at most post-feeding times. This may indicate that the NH_3-N rapidly released from urea was efficiently captured by the rumen microbes or rapidly absorbed across the rumen wall.

The results for total rumen VFA levels are difficult to explain as increasing levels were expected with increasing levels of beet pulp in the diet. However, the s.e. of the means were large and no definitive conclusions can be drawn. Moreover, the effect of diet on total rumen VFA was likely to be confounded with the effect of the level of total intake, which was highly variable among sheep (CV=0.25).

The molar proportions of acetic, propionic and n-butyric acid in the rumen were typical of forage-based diets (Thomas and Rook, 1977). The lower

proportions of acetic acid and the higher proportions of propionic acid at 2 and 4 h post-feeding compared to those at 0 and 7 h post-feeding were likely a result of the release of the more fermentable carbohydrates from the lucerne and/or beet pulp.

6.4.1.2. Intake and *in vivo* digestibility of the diets

An important finding from Experiment 8 is that the intake of straw OM increased linearly as the level of beet pulp in the diet increased. The increase of 0.5 g in straw OMI for each g of sugar beet pulp consumed is lower than that of 0.69 calculated from the results of Silva and Ørskov (1985). The reasons for this difference were probably similar to those already proposed for the differences in the *in sacco* degradation of straw. In the present experiment, the increase in straw OMI was probably a result of an increased degradation of straw in the rumen and/or a faster rate of passage of small straw particles through this organ . In Experiment 7, the *in sacco* degradation of straw in the rumen tended to increase as the level of sugar beet pulp increased. A faster rate of escape of straw particles was likely to occur to a small extent since the total level of intake increased from 0.7 (times the ME requirements of maintenance) in diet US-OBP to 1.1 in diet US-100BP.

Straw OMI was not significantly different among diets in Experiment 7 with 60-kg fistulated sheep fed the same diets as in the *in vivo* digestibility study (Experiment 8). This may be explained by the large variation in the intake data (CV=0.25) which increased the probability of not detecting real differences. Moreover, the sheep in this experiment were not blocked according to their straw intake during a pre-experimental period as were the sheep in Experiment 8. In this latter experiment blocks accounted for about 0.3 of the total variation in straw OMI (See Table 6.5A).

Although the *in vivo* digestibility of all chemical fractions in the diets increased linearly as the level of beet pulp increased, there was an indication of different responses with beet pulp levels below and above 667 g DM per kg total dietary DM (Diet US-67BP). Positive associative effects of straw, lucerne and beet pulp on total digestibility were apparent between diets US-OBP and US-67BP (Figure 6.4). For example, the calculated digestibility of straw OM, assuming the digestibilities for the pellets given in Table 6.11, increased from 0.454 in diet US-OBP to 0.519 in diet US-67BP. In diet US-100BP, the calculated digestibility of straw OM was similar to that in diet US-OBP (0.458 *v.* 0.454). Therefore, it appears that a combination of 2 parts of sugar beet pulp and 1 part of lucerne was optimum as a supplement for untreated barley straw under the conditions of this trial.

6.4.1.3. Crude protein digestibility and nitrogen balance

The increasing N balance associated with increasing levels of beet pulp in the supplement was probably a result of the increasing intakes of both N and ME. The N absorbed from the intestines may have been more efficiently utilized with diets promoting high intakes of digestible DM (Oldham *et al*, 1977). However, this is difficult to ascertain because the effects of increasing intakes of N and digestible DM were confounded.

The high CP digestibilities observed are in agreement with published results for straw-based diets containing urea (Bird, 1974; Oldham *et al*, 1977). The linear increase in CP digestibility from diet US-OBP to diet US-100BP was probably related to the increasing proportion of urea-N in the total N consumed. This urea-N may have been more efficiently utilized by the rumen microbes, as indicated by the similar levels of NH_3-N in the rumen for all diets despite an increasing intake of urea associated with increasing levels of sugar

beet pulp in the diet (Experiment 7).

6.4.2. Supplementation of ammonia-treated barley straw with lucerne and sugar beet pulp

6.4.2.1. *In sacco* degradation of straw and rumen fermentation

The effects of the level of sugar beet pulp on the *in sacco* degradation of straw were not consistent between the different incubation times and periods. However, at incubation times of 24, 48 and 72 h the responses below and above the level of 667 g sugar beet pulp per kg of pellets DM (Diet TS-67BP) appeared to be different. As in the trials with untreated straw, a combination of lucerne and sugar beet pulp in a ratio of 1:2 appeared to be optimum for maximum degradation of straw. The *in sacco* degradation of straw increased slightly from diet TS-OBP to diet TS-67BP and then decreased in diet TS-100BP. It is possible that a commensurate supply of digestible fibre and some slowly degradable protein (peptides and aminoacids) from the sugar beet pulp and/or the lucerne promoted a high microbial activity in the rumen. These trends were less evident for incubation period 2 than for period 1 (Table 6.7), indicating a possible short-term adaptation of the rumen microbes to the level of sugar beet pulp in the diet.

The lower *in sacco* degradation of straw in diet TS-100BP compared to the other three diets was associated with a lower rumen pH at all post-feeding times. In fact, this was below the critical level for fibre-degrading microbes (pH=6.1; section 2.4.3.1) for at least 8 h per day (See Figure 6.5). It is possible that the total amount of soluble sugars from sugar beet pulp and some fast-degrading hemicelluloses reduced the degradation of straw fibre not only through the negative effects of low pH on the activity of fibre-degrading bacteria but also by promoting substrate competition and preferences (Mould

et al, 1983). The growth and activity of the fibre-degrading bacteria was not likely to be reduced by competition with fast-growing sugar fermenters since fibre was the predominant substrate in the rumen. However, the fibre-degrading bacteria *per se* may have degraded the more easily-fermentable feed fractions leaving aside the more lignified fibre in the straw. Working with sheep, Fahmy *et al* (1984) found that the *in sacco* degradation of ammonia-treated straw started to decrease with levels of molassed sugar beet pulp above 350 g per kg of total DM. Such a level of sugar beet pulp was associated with higher levels of soluble sugars and higher rumen pH than those in the present trial. These differences are difficult to explain, but they may be partly associated with beneficial effects of small amounts of soluble sugars provided by the molassed beet pulp on rumen fermentation.

In general, the concentration of NH_3-N in the rumen decreased as the level of sugar beet pulp in the diet increased. This ocurred despite the small addition of urea to diets TS-67BP and TS-100BP and similar levels of N released from the treated straw in all diets. These results may be due to the following: a) the NH_3-N released in the rumen may have been more efficiently captured by the rumen microbes in sheep consuming diets with higher levels of beet pulp, where more fermentable OM was available and; b) the degradability of the CP in sugar beet pulp was likely to be lower than that in lucerne.

The total concentrations of VFA in the rumen were highly variable among sheep receiving diets based on treated straw (CV within diet and post-feeding time = 0.10-0.51). Moreover, the effects of the level of sugar beet pulp in the diet and of total level of intake were confounded. Therefore, no definitive conclusions can be drawn, but some trends will be discussed. The different

patterns of change in total rumen VFA with time post-feeding among diets (Figure 6.6) were probably associated with differences in the concentrations of soluble carbohydrates in lucerne and sugar beet pulp. There are more soluble sugars, starch and pectin and less hemicellulose in lucerne than in sugar beet pulp (Table 2.7). Ruminal degradation of sugars, starch and pectin is much faster than the degradation of the fibre components (Czerkawski, 1986; Sniffen *et al*, 1983). Therefore, the total concentrations of VFA in the rumen would be expected to increase faster after feeding diets containing higher levels of lucerne (TS-0BP and TS-33BP). The steady increase in total rumen VFA until 7 h post-feeding for diet TS-100BP was possibly a result of a high and steady degradation rate of the good-quality fibre in the sugar beet pulp. This is supported by the fact that the molar proportion of acetic acid increased with time post-feeding to a progressively larger extent as the level of sugar beet pulp in the diet increased (Table 6.8).

6.4.2.2. Intake and *in vivo* digestibility of the diets

The similar intakes of the sheep on diet TS-100BP compared to the other three diets was unexpected in view of the lower rumen pH and *in sacco* degradation of straw for diet TS-100BP. However, the s.e. of the means were large and very little of the total variation in straw intake was explained by the regression of straw OMI on sugar beet pulp OMI (Experiment 10). It is also possible that the effects of sugar beet pulp on rumen pH and *in sacco* degradation of straw were not strong enough to induce significant changes in intake, which is regulated by many factors, other than the degradation capacity of the rumen microbes (Baile and Forbes, 1974).

The changes in the digestibilities of the diets with level of sugar beet pulp were similar to those observed for diets based on untreated straw (Experiment

8); *i.e.* a positive associative effect of treated straw, lucerne and sugar beet pulp on total digestibility was apparent between diets TS-OBP and TS-67BP. This trend is consistent with the patterns of *in sacco* degradation of straw at 24, 48 and 72 h of incubation, which were discussed previously. Therefore, it appears that the supplement containing lucerne and sugar beet pulp in a ratio of 1:2 was also optimum when offered at 480 g DM per kg of total dietary DM in diets based on treated straw. However, for all the mixed diets the calculated digestibility of the treated-straw was lower than that of the same straw fed alone (Experiment 5, Chapter 4). For example, assuming digestibilities for the pellets given in Table 6.11, it can be calculated that the digestibility of the straw OM was reduced from 0.603 when fed alone to 0.543 for diet TS-67BP. This is likely to be a result of the faster rate of passage of digesta brought about by high levels of supplementation with pellets (See Chapter 5).

6.4.2.3. Crude protein digestibility and nitrogen balance

As the level of sugar beet pulp in the diet increased, the amount of digestible CP retained by the sheep remained similar. These results were unexpected, considering that the intake of digestible OM increased and that the total N intake was similar for all diets. Oldham *et al* (1977) showed that at similar N intakes, N balance in sheep increased as the intake of digestible DM from straw-based diets increased. The present results are largely unexplained.

6.4.3. *In vivo* digestibility of pellets containing lucerne and sugar beet pulp

The digestibilities of DM, OM, GE and NDF and the ME concentration in the sugar beet pulp pellets were within the range of published values (Kelly, 1983; Van Soest, 1982; Wainman *et al*, 1979; Table 2.6). The predicted digestibilities and ME concentrations for the lucerne pellets were also within the range of values published in the literature (*e.g.* Hunt *et al*, 1985; Soofi, Fahey, Berger

and Hinds 1982; Thomson and Cammell, 1979; Thomson, Beever, Coelho da Silva and Amstrong, 1972; Table 2.6). There was no indication of an associative effect of lucerne and sugar beet pulp on total digestibility in the pellets. Therefore, it can be assumed that the apparent positive associative effects for diets US-67BP and TS-67BP were probably due to associative effects of the pellets and the straw on total diet digestibility and/or straw intake.

6.4.4. General

The results from the *in vivo* digestibility trials indicate that replacing lucerne by sugar beet pulp in supplements for untreated or ammonia treated straw effectively increased the ME concentration of the diets. This resulted from a direct effect of the higher ME concentration of the sugar beet pulp and, to a lesser extent, from an apparently indirect effect of sugar beet pulp, whereby the intake and/or the digestibility of the straw was increased. This latter effect was more evident with untreated-straw diets than with treated-straw diets, where the degrading capacity of the rumen microbes may have been close to maximum because of the fermentable fibre made available through ammoniation of the straw (See Section 2.6.3).

With the levels of intake observed in Experiment 8, 40-kg sheep could obtain only about 0.7 of their ME requirements for maintenance (ARC, 1980) from diet US-0BP whereas diets US-67BP and US-100BP supplied 1.1 times the maintenance requirements. In Experiment 10 diets TS-0BP and TS-100BP supplied 1.3 and 1.7 of ME requirements for maintenance, respectively. Higher intakes of ME in relation to maintenance requirements would be expected with cattle, which have higher relative intakes of digestible nutrients from diets based on low-quality roughages (See Sections 2.6.1.2, 3.3.1 and 3.4).

The supplement containing 333 g of lucerne and 667 g of sugar beet pulp per

kg of total DM increased or maintained the intakes of untreated straw and also increased significantly the total intake of digestible nutrients. Therefore, such a supplement may be advantageous over grain supplements in common use, which decrease the intake and digestibility of roughages if they represent more than 0.3 to 0.4 of the total diet (See Section 2.4.3.1). The work of Ali (1985), done with the same feeds as those used in the present experiments supports this suggestion. With 35-kg sheep, the intake of untreated straw was reduced to a lesser extent and the total intake of DOM and ME increased to a larger extent by supplementing with a lucerne:sugar beet pulp supplement (1:1) than by supplementing with barley grain. With ammonia-treated straw, the total intakesof DOM and ME were similar with both supplements, but the straw intake was reduced to a larger extent by barley supplementation (Table 6.12). This indicates a better utilization of the straws and the supplements when lucerne and sugar beet pulp were used since the ME value of the barley is higher (about 12.9 MJ per kg) than the ME value of the lucerne:sugar beet pulp pellets (10.2 MJ per kg, from Table 6.11). It would be interesting to investigate whether the superiority of lucerne:sugar beet pulp supplements applies to a wider range of low-quality roughages and levels of supplementation.

TABLE 6.12 Intake and OM digestibility of the diets in sheep offered untreated or ammonia-treated barley straw supplemented with barley grain (B) or a lucerne: sugar beet pulp mixture (1:1) (LBP) (Adapted from Ali, 1985).

	Untreated straw			Treated straw		
	Supplement			Supplement		
	None	LBP	B	None	LBP	B
g supplement/kg, DM basis	0	530	430	0	542	539
OM digestibility, g/g						
Straw[1]	0.512	0.455	0.420	0.577	0.489	0.510
Total	0.512	0.603	0.605	0.577	0.622	0.669
Daily intake						
Straw OM, g	695	562	497	884	678	628
Total OM, g	695	1158	870	884	1443	1354
Total DOM, g	356	698	526	510	898	906
Total ME, MJ	4.91	9.94	7.36	7.52	12.95	13.35

1. Calculated using the digestibility values for lucerne and sugar beet pulp shown in Table 6.11 and an OMD of 0.805 for barley (Wainman *et al*, 1984).

6.5. CONCLUSIONS

a. Supplementation of untreated and ammonia-treated barley straw with pellets containing lucerne and sugar beet pulp in a ratio of 1:2 results in:

   - a slight increase in the degradation of straw in the rumen
   - an increase in, or maintenance of the intake and/or digestibility of the straws compared to the corresponding values in diets supplemented with beet pulp or lucerne alone
   - a significant increase in the intake of digestible nutrients

b. Diets based on untreated straw supplemented with pellets (320 g per kg of total diet, DM basis) containing lucerne and sugar beet pulp in a ratio of 1:2 may be suitable for maintenance in 40-kg sheep. Diets based on treated straw supplemented with similar pellets at 420 g per kg of total diet (DM basis) may be suitable for liveweight gains of about 60 g per day. It would be interesting to investigate further the suitability of the diets for larger ruminants.

c. The low intake of digestible nutrients that ruminants can obtain from cereal straws can be efficiently increased through supplementation with lucerne and sugar beet pulp. These feeds are rich in plant protein and digestible fibre which are more efficiently utilized through microbial degradation in the rumen than through enzymic digestion in the gastrointestinal tract of non-ruminants.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSIONS

The experiments carried out in this project were designed with the following principles in mind for the improvement of the utilisation of cereal straws by ruminants: a) inputs of energy and other resources used to increase the feeding value of the straws should be minimized; b) rumen fermentation should be maximized; and c) supplements to cereal straws should preferably be feeds that are more efficiently utlized through microbial fermentation in the rumen than through enzymic digestion in the gastrointestinal tract of non-ruminants.

7.1. INPUTS TO INCREASE THE FEEDING VALUE OF CEREAL STRAWS

There were two main reasons for applying minimum mechanical processing to the straws. Firstly, to prevent the addition of a high-energy cost to the costs already incurred by collection, baling, transport, storage and/or ammonia-treatment of the straw. Secondly, to develop straw-based diets applicable in conditions where mechanical processing is not cost-effective or not possible; *e.g.* in tropical areas where ruminants are offered cereal straws and other low-quality roughages as left on the field. The beneficial effects of mechanical processing of roughages on the intake of digestible nutrients and their efficiency of utilisation by ruminants (Greenhalgh and Wainman, 1972; Owen, 1978) were not overlooked. Mechanical processing implies costly machinery and high inputs of fossil-fuel energy which limit its use with roughages of low economic value (Shepperson, Marchant, Wilkins and Raymond, 1972; Walker, 1984). However, mechanical processing may be applicable to more valuable supplementary feeds.

Grinding and pelleting leguminous crops for feeding may not be justified for

some systems of animal production. However, pelleting is not strictly necessary when the legume forms only part of the total diet. In tropical areas where fossil-fuel energy is scarce and solar energy abundant, some form of manual or animal-powered crushing of the leafy fractions of leguminous trees and shrubs, after sun drying, could replace fuel-powered mechanical grinding. Such a physical treatment may be sufficient to prevent a high replacement of low-quality roughages by leguminous crops in the diet. The critical mean particle size of the comminuted legume forage above which such replacement occurs is a subject for further research.

The high energy cost of the oven-treatment of the straw with gaseous ammonia was realized before the experiments were undertaken. This method was used to ensure an effective treatment and a uniform material. Less energy-demanding methods using other sources of ammonia (*e.g.* urea, urine) and locally available resources will be more appropriate if costs of upgrading low-quality roughages are to be minimized (Sundstøl and Coxworth, 1984).

Diet selectivity was an important side effect of feeding coarsely shredded straws. In all the experiments, sheep selected the leafy fractions and refused the stemy fractions of the straw; the phenomenon was more evident with untreated straw. Similar observations were reported by Wahed and Owen (1986a, b) for sheep and goats offered untreated or ammonia-treated barley straws. In Experiment 1 (Chapter 3), it was shown that cattle were less selective than sheep. As indicated by Van Soest (1982), diet selection is an adaptative feeding habit of small ruminants to counterbalance their lower capacity to digest poor-quality roughages. Cattle, however, can also be selective. For example, in the experiment of May and Barker (1984) cattle apparently consumed more nutritious fractions of barley straw when this was

offered as a stubble on the field than when it was offered as bales in individual pens.

Diet selectivity has some important implications for practical and research purposes, especially with sheep. Firstly, sheep can be used as efficient separators of the leafy and stemy fractions of straws. On a different scale, this would have objectives similar to those of mechanical separation methods proposed for on-farm and industrial use (Davis *et al*, 1986; Rexen, 1978; Vind, 1984). The less digestible straw refusals can be used for several purposes. They can be used for bedding livestock or they can be burnt as household fuel where firewood is scarce (Verma and Jackson, 1984). In areas of the world where chemical treatment is only marginally profitable it may be beneficial to upgrade refusals only (Owen and Kategile, 1984).

A second implication of diet selectivity is that the *in vivo* digestibilities of some chemical fractions in the straw are over or underestimated, depending on the chemical fraction. This is particularly relevant to the digestibility of fibre. For example, if the straw refusals are higher in ADF than the straw as offered to sheep (Wahed and Owen, 1986a) the ADF digestibility will be overestimated as this is normally calculated using the ADF of the straw as offered. These effects are likely to contribute to the poor relationships between the *in vivo* and the *in vitro* digestibility of these feeds and also between their *in vivo* digestibility and certain fractions of the DM; *e.g.* NDF and ADF (Barber *et al*, 1984). This is not surprising since the digestibility of two rather different materials is measured by *in vivo* and *in vitro* methods; *i.e.* the straw fractions selected by the sheep and a ground sample of the straw as offered.

7.2. MAXIMIZING RUMEN FERMENTATION

Rumen degradable protein (RDP), good-quality fibre, minerals, vitamins and growth factors (*e.g.* preformed aminoacids and branched chain VFA) were supplied in the feeds used in these experiments with the aim of maximizing the fermentative capacity of the rumen (Preston and Leng, 1984). Whether a maximum was reached cannot be assured with the results obtained, but there were indications of an improved activity of the fibre-degrading microbes. This was achieved through supplementation with lucerne and sugar beet pulp which are more suitable for ruminants than for non-ruminants and, certainly, unsuitable for humans.

Supplementation with lucerne pellets was successful in that it increased the intake of digestible nutrients and also maintained the intake and microbial digestion (*in sacco*) of the straws. Several factors appeared to have contributed to the low or zero replacement of straw by lucerne in the diets. Firstly, the low bulk volume of the lucerne pellets. Secondly, the adequate supply of nutrients to the rumen microbes. Thirdly, the apparent lack of effect of lucerne supplementation on the size reduction of straw particles in the rumen. However, not all the results of supplementing with lucerne pellets were beneficial. It can be calculated that the *in vivo* digestibility of the straws, which is influenced by digestion and passage, was lower in diets with high levels of lucerne than in unsupplemented diets. However, the final outcome on the total intake of digestible nutrients was far better than that obtained by other workers with low-quality roughages supplemented with high-quality legumes and grasses in the long or coarsely chopped forms (Figures 2.10 and 2.11).

The present results are particularly relevant for tropical areas where

leguminous crops are plentiful, some of which (shrubs and trees) have a high nutritive value throughout the year (Ibrahim, 1981; National Academy of Sciences, 1979). A further benefit of using leguminous crops as supplements to low-quality roughages is that they contain high levels of protein, especially RDP. In fact, the rumen degradability of the CP in two representative tree legumes from the tropics (*Leucaena leucocephala* and *Gliricidia sepium*) in the rumen of sheep fed lucerne pellets alone (Experiment 2) was about 0.7. RDP from leguminous crops can replace RDP from commonly used sources of NPN, like urea, which is scarce, expensive and more commonly used as a fertilizer for crops yielding food for humans in many tropical areas.

The beneficial effect of supplementing diets based on straw and lucerne with sugar beet pulp was twofold: a direct increase of ME in the total ration and some improvement in the digestibility and/or the intake of the straw. The latter effect was apparently associated with an enhanced activity of the fibre-degrading rumen microbes sustained by the digestible fibre in the sugar beet pulp. Similar results obtained by Silva and Ørskov (1985) with sheep fed barley straw and sugar beet pulp were also associated with stimulatory effects of highly-digestible fibre on rumen fermentation. Gutierrez, Elliot, Harrison and Preston (1983) (Cited by Preston and Leng, 1984) found that a supplement of fresh grass added to a basal diet of ensiled sisal pulp (*Agave fourcroydes*) improved the *in sacco* degradation of the sisal cellulose in the rumen of sheep. They attributed these results to stimulatory effects of the good-quality fibre and other factors in the fresh grass on rumen fermentation. A synergistic effect of high-quality fibre and other factors may be the reason why a supplement containing two parts of sugar beet pulp and one part of lucerne apparently promoted a higher intake and/or digestibility of straw than did supplements containing lucerne or sugar beet pulp alone (Chapter 6).

Preformed aminoacids, minerals and other micronutrients which are essential or stimulatory for growth of fibre-degrading microbes were provided by lucerne and sugar beet pulp (Section 2.4.3.2). Moreover, with a supplement containing lucerne and sugar beet pulp in a ratio of 1:2 a well balanced release of fermentable energy could have occured. In fact, fermentable energy originated from fractions having a wide range of degradation rates; *e.g.* soluble sugars, pectin and structural carbohydrates (Bailey, 1967; Sniffen *et al*, 1983).

The growth and activity of the fibre-degrading bacteria may have been improved in the experiments through the use of a slow-release source of NPN or minerals (Bartley and Deyoe, 1981; Meggison *et al*, 1979; Thomsen, *et al*, 1978). Whether these sources are effective with the type of diets in the present work requires further investigation.

Although supplementation with sugar beet pulp increased the ME value of the diets, its use requiresthe use of more urea to supply the RDP requirements of the rumen microbes. This means that the benefits of replacing urea RDP by legume RDP may be partly offset. Alternative sources of NPN, *e.g.* poultry excreta, may help to reduce the amounts of urea required in the diets. However, it would be difficult to replace significant quantities of urea (on a N-concentration basis) in diets containing high levels of sugar beet pulp and lucerne.

7.3. POTENTIAL OF DIETS BASED ON CEREAL STRAWS, LUCERNE AND SUGAR BEET PULP FOR DIFFERENT CLASSES OF RUMINANT LIVESTOCK

The ultimate objective of improving the utilisation of cereal straws by ruminants is to cheaply increase the total intake of digestible nutrients and animal performance. The intakes of ME determined with sheep in the present work were used to examine the potential of the diets studied. These ME

intakes were compared with the ME requirements for maintenance, and used to predict animal performance (ARC, 1980). It should be emphasized that the calculations were done to present an integrated view of the results obtained and that the limitations of intake results from short-term trials were recognized (Greenhalgh and Wainman, 1972; Owen and Kategile, 1984). It was also recognized that although predictions of performance are useful to give an indication of the potential of the diets they are often different from actual results (Greenhalgh, 1984).

7.3.1. Growing lambs and non-lactating pregnant ewes

The results from the *in vivo* digestibility trials using 35-kg sheep were used to examine the potential of the diets for growing lambs whereas the results from the *in sacco* degradation trials with 60-kg sheep were used to assess the possible use of the same diets for pregnant ewes.

With 35-kg wethers offered untreated straw and increasing levels of lucerne, the intake and ME values of the diets were very low (Experiment 3). The ME values of the diets in this experiment were used in the calculations because no other information was available, but the intake results were not used. The DM intakes used to calculate ME intakes were those observed in Experiment 8, with 35-kg sheep offered untreated straw and pellets containing lucerne and/or sugar beet pulp. The ME values of the diets observed in the *in vivo* digestibility trials were used to calculate the ME intakes of 60-kg sheep in the respective *in sacco* degradation trials. The results are presented in Figure 7.1, together with estimates of the ME requirements for maintenance (ARC, 1980). These requirements differ slightly from those referred to in previous discussions (Chapters 4 and 6) because the ME expenditure for activity was added to the ME expenditure for fasting heat production.

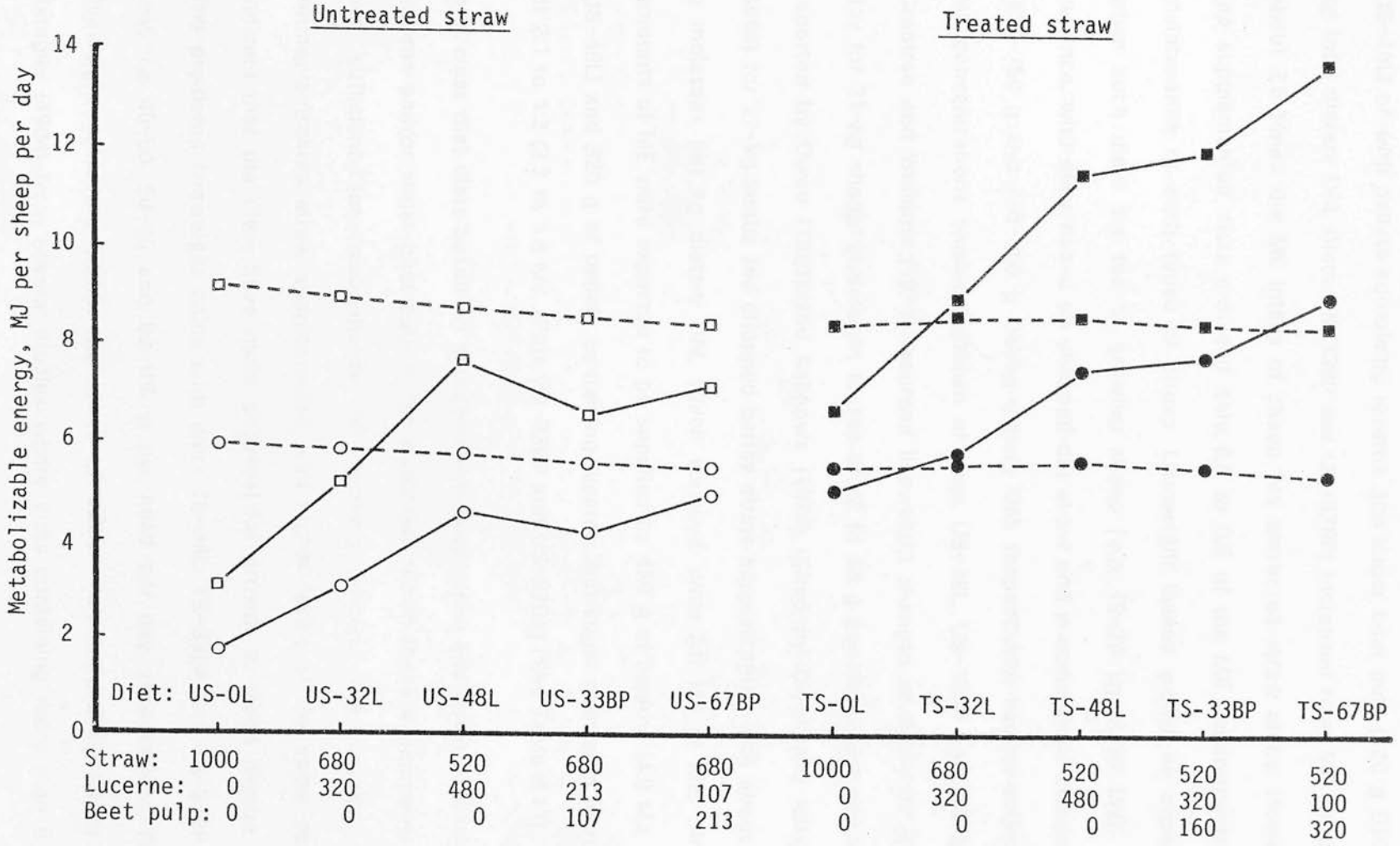

FIGURE 7.1 ME intakes (——) and ME requirements for maintenance (---) of 35-kg (o, ●) and 60-kg sheep (□, ■) offered diets containing barley straw, lucerne and sugar beet pulp (g/kg, DM basis).

Supplementing untreated straw with lucerne pellets at the highest level (Diet US-48L) or with pellets containing lucerne and sugar beet pulp (320 g DM per kg total dietary DM, Diets US-33BP and US-67BP) increased total ME intake to about 2.5 times the ME intake of sheep fed untreated straw alone. However, the supplemented diets provided only 0.8 to 0.9 of the ME requirements for maintenance of both types of sheep. Liveweight losses would be expected when such diets are fed to growing sheep (*e.g.* 15-20 kg initial LW). For instance, with diets based on chopped oat straw and a commercial compound (620-750 g and 250-380 g DM/kg dietary DM, respectively) having estimated ME concentrations similar to those of diets US-48L, US-33BP and US-67BP, Crabtree and Williams (1971) recorded liveweight changes of 8 to -17 g per day for 21-kg sheep. Liveweight losses of 18 to 68 g per sheep per day were reported by Owen (1981) and Kalinows (1974) (Cited by Owen and Kategile, 1984) for 27-kg lambs fed chopped barley straw supplemented with about 300 g molasses per kg dietary DM, which supplied *circa* 3.6 MJ of ME. Similar amounts of ME were expected to be supplied by 480 g of lucerne (4.0 MJ, Diet US-48L) and 320 g of pellets containing lucerne and sugar beet pulp in ratios of 2:1 to 1:2 (3.2 to 3.6 MJ, Diets US-33BP and US-67BP) (See Table 6.11).

It is clear that diets based on shredded untreated straw and pellets containing lucerne and/or sugar beet pulp in the quantities fed in these experiments are not sufficient for maintenance of growing sheep. The results with ammonia-treated straw supplemented with higher levels of the same pellets indicate that the diets have more potential for growth in sheep (Figure 7.1). The predicted liveweight gains with diets TS-48L, TS-33BP and TS-67BP are low; *i.e.* 40-50, 50-60 and 90-100 g per head per day, respectively. These liveweight gains are within the range of values summarized by Owen and Kategile (1984) from several studies where diets containing more than 0.5 of

chopped, alkali-treated low-quality roughages were fed to growing sheep. Higher liveweight gains have been obtained with treated straw diets similar to those of the present work when the straw was processed mechanically (Greenhalgh, Pirie and Reid, 1976; Saxena, Otterby, Donker and Good, 1971) and/or the diets were further supplemented with feeds of low rumen degradability (Abidin and Kempton, 1981). Nevertheless, it would be difficult to obtain liveweight gains exceeding about 150 g per head per day in growing sheep fed diets with over 0.5 of alkali-treated low-quality roughages in the total diet (See reviews by Greenhalgh, 1984 and Owen and Kategile, 1984). As indicated previously, mechanical processing of roughages of low economic value has limited potential where fossil-fuel energy is scarce. Supplementation of diets based on straws, lucerne and sugar beet pulp with sources of rumen undegradable protein and/or energy to improve lamb performance deserves further investigation.

The ME intakes estimated with 60-kg wethers (Figure 7.1) may be useful to examine the potential of the diets for non-lactating pregnant ewes, assuming that the intakes of straw are similar for the two types of animals. In fact, the average daily intakes of untreated and treated straw by the 60-kg wethers (27.5 and 36.5 g DM/kg $LW^{0.75}$, respectively) were close to the range of values reported for pregnant-ewes (75-85 kg LW) offered long untreated or ammonia-treated barley straw at levels of 0.5 to 0.8 of the total diet (16-34 g DM/kg $LW^{0.75}$ and 27-35 g/kg $LW^{0.75}$, respectively) (Table 15.3 of Owen and Kategile, 1984; Orr, Treacher and Mason, 1985). It is unlikely that the ME requirements of pregnant ewes can be satisfied in full with any of the untreated-straw diets shown in Figure 7.1. On the other hand, the estimated ME requirements of 60-kg ewes carrying twin lambs (8 kg birth weight) up to about 119 days of pregnancy are likely to be satisfied in full with diets TS-48L,

TS-33BP and TS-67BP; diet TS-67BP may be adequate up to 126-133 days of pregnancy. However, if the ewes are in good body condition in late pregnancy and some mobilisation of body reserves is acceptable, then the latter diet should be adequate until lambing. For ewes carrying single lambs, diets TS-46L, TS-33BP and TS-67BP supply the full estimated energy requirement right up to lambing.

7.3.2. Growing cattle and non-lactating pregnant cows

Several authors have shown that non-lactating cows and growing and finishing beef cattle can attain satisfactory performance when fed diets containing long or coarsely chopped cereals straws (untreated or chemically-treated) representing 0.5 to 0.7 of the total diet (*e.g.* Andrews, Escuder-Volonte, Curran and Holmes, 1972; Mira, Kay and Hunter, 1983a,b; Owen, 1984; Pirie and Greenhalgh, 1978; Strickland, 1984). Growth rates varied widely among the different experiments due to many factors, including straw quality and the level and nature of other components of the diet. It is likely that some of the diets studied in the present work can be suitable for growing cattle. No attempt was made to predict performance of cattle based on the intake and digestibility results obtained with sheep because it would have implied too many assumptions. The diets would be expected to give relatively better performance in cattle than in sheep considering that the former can achieve higher intakes relative to body size and can digest low-quality roughages to a larger extent (Mertens and Ely, 1982; Playne , 1978a,b; Prigge *et al*, 1984). Even higher intakes of digestible nutrients may be achieved by native tropical cattle which are genetically adapted to consume bulky low-quality roughages for long periods (Mould, Saadullah, Haque, Davis, Dolberg and Ørskov, 1982).

7.4. GENERAL CONCLUSIONS AND FURTHER RESEARCH

Supplementing untreated or chemically-treated barley straw with lucerne and/or sugar beet pulp, or similar feeds, helps to overcome the major constraint in the utilisation of the straws by ruminants, *i.e.* the low intake of digestible nutrients. This can be generally achieved with little or no reduction in the digestibility and intake of coarsely shredded straws. Two main factors are associated with these results: a)feeding the supplements, especially the lucerne, ground and pelleted to prevent high replacement effects due to physical limitation of the rumen; b) promoting a favourable rumen environment for maximum degradation of the major energy-yielding substrate of the straw; *i.e.* the cell wall.

The potential of diets based on straw, lucerne and/or sugar beet pulp for ruminants depends on the type of livestock and the target level of production. Diets based on untreated straw may be appropriate for just maintenance in sheep and moderate levels of production in cattle, especially if some sugar beet pulp or similar feed is included in the supplement. Ammonia-treatment can effectively increase the nutritive value of the straw. This, however, should not be overemphasized as optimum supplementation of selected good-quality straws fed in the untreated form (or other low-quality roughages) may be a more appropriate strategy in the future.

Performance trials with sheep and cattle of adequate duration and replication should be done to test the potential of some of the diets studied in the present work. The effect of supplementing the diets with sources of rumen undegradable nutrients on performance should be also studied. More research should be undertaken to elucidate the nature of the stimulating effect of good-quality fibre and other factors on intake and digestibility of low-quality

roughages; the rôles of the fibre-degrading activity of the microbes and the influence of feed comminution in the rumen in the control of this phenomenon should be considered.

As applied to the feeds in the present work, chemical treatment of the straw and mechanical processing of the supplements have little scope in tropical areas where chemicals are costly and fossil-fuel scarce. Less energy-demanding processing methods with locally available resources should be used in these areas. On the other hand, the principles of supplementation adopted in the present work are applicable worldwide. They imply a judicious use of the ruminant as a converter of fibrous roughages and by-products into useful products without competing with non-ruminants, including humans, for food sources.

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GONZALEZ-VARGAS, D.; LEWIS, M. and WILSON, P.N. (1986). The effect of lucerne supplementation on the intake of straw and the digestion kinetics of straw and lucerne. *Anim. Prod. 42*, 472. (Abstract).

132. THE EFFECT OF LUCERNE SUPPLEMENTATION ON THE INTAKE OF STRAW AND THE DIGESTION KINETICS OF STRAW AND LUCERNE

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In two experiments 12 mature sheep of 60 (s.e. 1·2) kg live weight (LW), fitted with rumen cannulae, were fed either ammonia-treated straw (TS) or untreated straw (US) plus urea. The straw was supplemented with lucerne pellets at levels of 0, 0·16, 0·32 and 0·48 of total dietary dry matter (DM). Dacron bags containing samples of the straws or lucerne were incubated in the rumen for 5, 11, 24, 48 and 72 h. Lag time (t_0, h), potential digestibility (PD, g/g) and digestion rate (K, per h) were estimated for straw and lucerne DM. Straw intakes (g DM per kg $LW^{0·75}$) were similar ($P < 0·05$) at all levels of lucerne supplementation (29·7 (s.e. 3·5) and 35·8 (s.e. 3·0) for US and TS). Total intake (g DM per kg $LW^{0·75}$) was significantly increased ($P < 0·05$) at the 0·48 lucerne level, 57·3 (s.e. 2·8) (US) and 63·6 (s.e. 1·7) (TS), compared to straw alone, 33·2 (s.e. 2·1) (US) and 35·2 (s.e. 4·3) (TS). Differences among diets were largely non-significant ($P > 0·05$) for t_0, PD and K. Means for t_0 were 5·4 (s.e. 0·2) (US) and 4·7 (s.e. 0·7) (TS). Mean potential digestibilities were 0·58 (s.e. 0·03) (US) and 0·73 (s.e. 0·08) (TS) and mean K values 0·033 (s.e. 0·005) (US) and 0·040 (s.e. 0·009) (TS). For lucerne incubated in the rumens of sheep fed US: t_0 = 5·9 (s.e. 0·5), PD = 0·71 (s.e. 0·002) and K = 0·094 (s.e. 0·022), with similar values in sheep fed TS. It was concluded that lucerne pellets could be included up to 0·48 in straw diets without affecting the digestion kinetics of the dietary components or straw intake while increasing the total intake of energy and protein.

APPENDIX

TABLE 2.1A Examples of wet sieving techniques reported in the literature for size fractionating of particles in feed, digesta and faeces

Sample size, g DM	Sieve diameter cm	Sample sizes: screen area g DM/cm^2	Aperture size of sieves used, mm, (Side size of square apertures, or diameter of circular apertures)	Sieving time, mm\	Sieving procedure	Reference
7.5	11	0.08	6.8, 3.2, 2.0, 1.00, 0.70, 0.50, 0.25	Variable	Sample placed on coarsest sieve which was fitted at the bottom of a cylinder. The cylinder was dipped into a beaker containing 2.5 l of water and the slurry stirred manually while the cylinder was moved vertically five times. The particle matter retained on the sieve was transferred to a dish for drying. The slurry left in the beaker was then sieved with the successively smaller sieves.	Dixon and Milligan (1985)
Variable	9	Variable	2.4, 1.20, 0.60, 0.30, 0.075	2	Column of five sieves in descending order with the coarsest sieve on top. Samples placed on the top sieve and washed down by a jet of water to an outlet at the bottom while the set of sieves was rotated and the particles on each sieve spreaded by soft rubber brushes.	Evans *et al* (1973)
N.R.	20	-	2.06, 1.12, 0.71, 0.43, 0.31, 0.21 0.16	Variable	Column of sieves as above. Sample poured on the top sieve and washed with a strong stream of water until no more particles could go through the mesh. The process was then repeated on successively smaller sieves.	Pearce (1967)
10	20[1]	0.03	4.75, 2.36, 1.18, 0.6, 0.3, 0.15 [1]Assumed to be that recommended by ASAE (1967)	20	Column of sieves as above, immersed in a tank of water. Sample placed on the top sieve and the column shaken vertically at an oscillation distance of 6.5 cm and at a frequency of 40 strokes/min. Water level in the tank adjusted so that at the botton of the stroke water flowed into the top sieve and at the top of the stroke water flowed through and out of the sieve column	Poppi *et al* (1980)
2-3	14.6	0.015	4.0, 2.0, 1.18, 0.6, 0.425, 0.3, 0.15	60	Column of sieves in order of descending mesh size. Sample placed on the top and washed down in a closed water circuit with a fixed amount of liquid, without shaking.	Jones and Moseley (1977)
0.5	6.3	0.016	1.2, 0.6, 0.3, 0.15, 0.075, 0.038	12	Column of sieves as above. Wet NDF sample placed on top sieve and sprayed with water while vibration and vacuum were applied to the air-tight system. The process was repeated for each successively smaller sieve for 12 min, at 3-min intervals; with hand-stirring of the sample between intervals	Allen *et al* (1984)
2-3	20	0.008	8.0, 6.3, 4.0, 2.5, 1.6, 0.8, 0.4, 0.16, 0.1	20	Column of sieves as above, mounted on an electromagnetic shaker, the lid of which had an inlet spray and the bottom pan an oulet pipe Vibration amplitude of 3 mm at 3000 cycles/min. Total amount of water used: 4.5 l. 15 min vibration applied at 5 min intervals.	Grenet (1984)

TABLE 2.2A Means and ranges for chemical composition of the dry matter in cereal straws.

Crop species	MJ/kg	g/kg											mg/kg			Reference Observations
	GE	Total N	CF	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Oats		6.1 5.9–6.6	419 414–420													Kernan *et al*,1979 n=3
	21.1 20.7–21.7	6.2 1.4–7.5		803 792–819	548 538–559	112 82–146	47 39–62	1.1 0.7–1.5	0.8 0.4–1.1	0.6 0.6–0.7	5.2 1.2–8.1	7.9 5.7–10.7				Pearce *et al*, 1979 n=3
		7.3 4.5–10.9														White *et al*, 1981 n=20
		5.1 2.7–7.2	410 339–451				75 41–167									Anderson, 1978; n=13 for N; n=10 for CF and ash
				767	461	53	67									Sundstøl *et al*, 1978a; n=1
	17.8 17.7–18.1	4.2 3.8–4.4	424 411–437													Horton and Steacy, 1979 n=3
	17.6	3.5	406				58									Horton, 1978; n=1
	18.6	6.2	426				54									Crabtree and Williams, 1971; n=1
		5.6				115	45	1.1	0.7	0.7	3.5	8.8				Mulholland *et al*, 1974; n=1
	18.5 18.5–18.6	6.9 5.9–7.9	371 371–371	784 781–787	512 503–521	68 68–69	60 58–62	2.7 2.6–2.8	1.7 1.4–2.4	0.9 0.7–1.1	1.6 0.7–2.6	13.0 10.4–15.7	6 5–7	22.0 18–27	25 22–28	Wainman *et al*, 1984 n=2

Table 2.2A Contd.

Crop species	MJ/kg	g/kg											mg/kg			Reference Observations
	GE	Total N	CP	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Barley		7.8 6.7–8.6	419 407–428													Kernan *et al*,1979 n=5
	20.1 18.5–20.7	4.7 4.0–6.4		773 756–799	536 502–560	80 51–97	68 56–92	1.9 1.3–2.4	0.6 0.4–0.8	0.9 0.6–1.1	1.4 1.0–2.2	4.3 3.7–5.0				Pearce *et al*, 1979 n=5
		8.2 5.9–11.2														White *et al*, 1981 n=19
		7.8 5.9–13.8		770 717–811	521 470–549	69 58–80	90 78–105		0.6 0.4–1.0							Erickson *et al*, 1982 n=37 Location 1 Year 1
		9.9 8.3–12.6		666 606–712	493 457–520	62 53–71	140 125–158	5.8 4.3–7.8	2.0 1.5–3.0	2.0 1.4–3.1						Erickson *et al*, 1982; n=31 Location 2 Year 2
		7.0 3.4–11.2	468 401–503				61 32–132									Anderson, 1978, n=13 for N, n=7 for CF, n=8 for ash

Contd. Table 2.2A

Crop species	MJ/kg	g/kg											mg/kg			Reference Observations
	GE	Total N	CP	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Barley				774 768–780	481 465–497	53 51–55	61 61–61									Sundstøl *et al*, 1978 n=2
		6.4 6.3–6.5	509 506–543				57 57–58									Palmer, 1976 n=19
	17.1 16.3–17.6		353 298–383													Horton and Steacy, 1979; n=3
		5.4 4.3–6.9			501 492–507		31 28–33									Mira *et al*,1983a,b n=3
	17.5			812	534	82	67									Abidin and Kempton, 1981; n=1
	18.5 17.9–18.9	7.4 5.6–8.4	410 376–431	827 788–858	534 524–555	77 70–89	54 41–70	3.0 2.4–3.9	1.3 1.0–1.5	0.5 0.4–0.7	0.8 0.7–1.0	11.0 7.9–14.2	5 5–5	25 20–32	23 16–36	Wainman *et al*, 1984 n=4

Table 2.2A Contd.

Crop species	MJ/kg	g/kg											mg/kg			Reference Observations
	GE	Total N	CF	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Wheat		5.8 5.3–6.2	432 404–448													Kernan *et al*,1979 n=3
	20.2 ?	4.2 2.4–7.8		796 756–830	571 525–611	92 81–104	66 31–97	1.8 1.0–3.1	0.8 0.3–1.2	0.6 0.4–0.8	0.4 0.1–0.8	6.3 3.5–8.8				Pearce *et al*, 1979 n=16
		5.4 3.0–6.9														White *et al*, 1981 n=20
		4.6		763	539	101										Mudgal *et al*, 1980 n=1
		6.6 4.4–8.5	411 379–431				102 75–142	1.5	0.4	1.0		16.1	3	–	37	Anderson, 1978; n=4 for N; n=3 for CF, ash; n=36 for minerals
		4.2			496											Kernan and Coxworth, 1981 n=1
	17.6 17.6–17.6	4.0 3.5–4.9	437 419–451													Horton and Steacy, 1979 n=3
	17.6	3.4	411				72									Horton, 1978 n=1
	18.2 18.1–18.4	6.2 6.0–6.4	428 411–446	823 796–851	575 549–602	85 79–91	71 68–75	2.6 2.5–2.8	0.7 0.6–0.9	0.4 0.4–0.5	0.3 0.2–0.4	11.9 9.5–14.3	3.5 3–4	21 20–22	14 12–17	Wainman *et al*,1984 n=2

Contd. Table 2.2A

Crop species	MJ/kg	g/kg											mg/kg			Reference Observations
	GE	Total N	CF	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Rice		7.8 5.1–12.2	317 279–340				149 131–175	5.3 2.5–6.7	3.1 1.5–5.4		2.0 1.4–3.0	4.1 2.5–8.1				Devasia *et al*,1976 n=11
		3.5		742	510	62										Mudgal *et al*, 1980 n=1
		4.7	364				135									Jayasuriya, 1979 n=1
		7.2	350			45	165	1.9	1.0	1.1		12.0	5.0		400	Jayasuriya, 1983 n=1
	15.9	6.3	334				251	0.9	1.2							Moran *et al*,1983 n=1
	16.7	8.7	346	747	459	58	106									Yoon *et al*, 1983 n=1

TABLE 2.3A Means and ranges for chemical composition and ME concentration of some highly digestible fibrous feeds.

Feed species	MJ/kg DM	g/kg DM											mg/kg DM			Reference Observations
	ME	CP	CF	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Lucerne (dried)																
M. Sativa	–	180	–	–	268	–	–	18.5	2.3	4.1			8.2	21.6	24	Christensen and Cochran; 1983; n=85
	9.2	189	262	450	350	110	106	15.2	2.5	3.2	1.1	26	11	21	34	National Research Council (NRC); 1984.
Cut 1	11.3	267	186	351	263	64	91	16.0	3.0	3.8	2.0	16	7	32	32	Rowett Research
Cut 3	10.4	227	203	372	266	69	93	20.0	2.0	4.0	3.0	11	8	22	32	Institute; 1976
Cut 2	8.3	194	278	480	371	92	112	15.0	3.0	3.0	2.0	12	8	30	61	n=2 for each cut
Red clover hay																
T. pratense	8.3	160	288	560	360	100	85	15.3	2.5	4.3	1.9	16	11	17	73	NRC; 1984
Ladino clover hay																
T. repens	10.3	220	212	360	320	70	101	13.5	3.1	4.8	1.3	26	10	17	95	NRC; 1984
Leucaena[1]																
L. leucocephala	–	195	220	–	–	–	87	20.0	1.6	4.0	0.8	20	–	23	45	Chadhokar; 1982
Gliricidia[1]																1. Dry leaves harvested every three months.
G. maculata	–	228	168	–	–	–	122	24.4	1.7	5.8	0.9	23	–	22	60	
Leucaena																
Leaves		239	134				88	27.9	2.4							Akbar *et al*, 1985
Leaves + stems	8.1	214	171				92	11.8	1.7							(Means from Tables given by the authors)
Leaves + stems							113	22.2	2.4	–	0 3	11	26	69	115	ME for cattle, from Wahyumi *et al*, 1982

Table 2.3A Contd.

Feed species	MJ/kg DM	g/kg DM											mg/kg DM			Reference Observations
	ME	CP	CF	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Sugar beet pulp																
Unmolassed	11.2	97	198	540	330	20	54	6.9	1.0	2.7	2.1	2.0	14	1	38	NRC, 1984
Molassed	11.5	101	165	440	250	30	61	6.1	1.0	1.6	5.3	17.8	16	2	27	
Unmolassed	11.6	101	204	480	288	32	60	8.0	1.9	6.5	0.6	6.0				Kelly, 1983; n=1 for unmolassed; n=9 for molassed
Molassed	12.5	129	124	294	177	21	83	5.9	0.7	0.1	3.7	17.4				
Unmolassed[3]	12.3	104	196	557	278	28	58									MAFF, 1982; 3.(Fresh)
Molassed	13.2	122	125	297	176	18	84	6.4	0.7	0.9	6.3	14.7	13	20	41	Wainman *et al*, 1979
	11.2	131	118	271	164	22	84	5.5	0.6	1.3	6.2	16.8	11	15	30	
	12.2	139	129	318	188	27	75	5.1	0.6	0.8	5.0	14.7	13	17	18	
	12.9	132	124	293	176	17	78	6.2	0.7	1.2	3.7	18.1	12	26	40	
	13.6	127	119	290	175	23	92	7.6	0.6	1.0	6.1	16.8	20	25	28	
	11.8	131	120	286	180	22	86	5.0	0.6	1.1	3.8	18.8	13	30	41	
	13.0	134	121	291	182	22	82	5.7	0.6	0.9	4.4	17.1	17	22	28	
	12.8	131	128	310	190	24	76	5.2	0.6	0.9	4.8	16.5	12	22	22	

Table 2.3A Contd.

Feed species	MJ/kg DM	g/kg DM											mg/kg DM			Reference Observations
	ME	CP	CF	NDF	ADF	ADL	Ash	Ca	P	Mg	Na	K	Cu	Zn	Mn	
Dried citrus pulp																
Mixed		67	134				51	14.3	1.1	1.2	1.0	10.9	6	10	6	Ammerman, 1973
Mixed	11.3	72		272[2]	266[2]		75	19.0	1.1	1.2						MAFF, 1985 [2]Ashed
	12.3	71		235	228		72	19.0	1.2	1.3						
Mixed	12.4	67	127	230	220	30	66	18.4	1.2	1.7	0.9	7.9	6	15	7	NRC, 1984
Orange		50	82		130	15	31									Martinez-Pascual and Fernandez-Carmona, 1980a
		55	94		160	10	35									
		60	109		191	16	37									
		65	93		165	13	33									
Satsuma		59	74		106	12	39									
Tangerine		80	100		174	21	40									
Grapefruit		71	98		168	19	38									
Lemon		71	109		168	18	38									
Orange	12.4	75	85				37	6.2	1.0							Ross, 1981
Tangerine	–	71	96				44	13.7	1.2							
Grapefruit	11.1	69	131				53	13.3	1.6							
Lemon	10.9	69	148				53	–	–							
Lime	8.9	65	152				51		–							
Orange		72	122				50	18.1	2.7	1.2	0.3	7.0	14	12	13	Lanza and Messina, 1979
Lemon		89	189				50									

TABLE 3.1A Composition of the mineral and vitamin supplement used in all experiments.

	g/kg		mg/kg
Calcium	200	Iron	6000
Phosphorus	150	Cobalt	150
Magnesium	50	Manganese	4400
Sodium chloride	100	Zinc	1800
		Iodine	80
		Selenium	12
		Copper*	1500
Vitamins	i.u./kg		
A	500.000		
D	100.000		
E	500		

N.B. The required amount of a mixture of this supplement and sodium sulphate at a ratio of 2:1 was offered to the animals at each feeding.

* For cattle only.

APPENDIX 3.2A
Determination of rumen NH_3-N
Reagents

a. Saturated potasium carbonate

b. N/10 HCl

c. N/100 HCl

d. Mixed indicator: 0.033 g bromocresol green + 0.066 g methyl red dissolved in 100 ml ethyl alcohol.

e. Boric acid indicator: 5 g boric acid weighed in a 1000 ml flask and iniatially dissolved with 200 ml alcohol and mixed with 700 ml distilled water. 10 ml mixed indicator and a few drops of 0.01 NaOH added to acquire the desired end-point (pale pink), before making up to 1000 ml. Working solution obtained by 50/50 dilution with distilled water.

f. Standard ammonium sulphate (About 0.2-0.25 g/500 ml distilled water)

Sample preparation

a. Rumen liquour was filtered through four layers of muslim.

b. 5 ml of this filtered fluid was then pipetted into a 25 ml volumetric flask containing 5 ml of N/10 HCL.

c. The flask was made up to the mark with distilled water and filtered through a Whatman No. 2V paper.

d. 1 ml of this filtrate was used in the Conway diffusion test.

Conway test

a. The required Conway dishes and lids were greased to effect a gas-tight seal between them. By slightly displacing the lid to gain access to the dish, the test reagents were added as follows: 1 ml of boric acid indicator into the centre well, 1 ml of the prepared sample filtrate and 1 ml of saturated K_2CO_3 into the outer chamber ensuring that these were not mixed until the lid was replaced.

b. The dishes were gently rotated to mix the sample and the K_2CO_3 and initiate the release of NH_3. This diffused into the boric acid indicator which was acid-titrated (N/100 HCL) after two hours.

c. A standard sample of amonium sulphate was run with every batch of rumen liquor samples, *i.e.* every time post-feeding.

APPENDIX 3.3A

Determination of rumen VFA

Reagents

a. Metaphosphoric acid solution (MAS): 0.25 metaphosphoric acid in 5N H_2SO_4

b. VFA standards containing 2 ml of MAS and the following approximate weights of each acid (mg per 100 ml):

Acetic	180
Propionic	126
Iso-Butyric	18
N-Butyric	90
Iso-Valeric	18
N-Valeric	18
N-Hexanoic	18

Sample preparation

a. Rumen liquour was filtered through four layers of muslim.

b. 10 ml of this filtrate were pipetted into a stoppered glass test tube and 2 ml of MAS added. The tube was shaken well and placed in a fridge (about 5 °C) for 30 mins to favour protein precipitation.

c. The liquid phase was transfered to centrifuge tubes and spun for 30 mins at 3500 r.p.m. The centrifuge tubes were placed in a the fridge for 30 mins.

d. The supernatant was then carefully decanted and stored at -4 °C before analysis by Gas-liquid Chromatoghapy (g.l.c.)

g.l.c. analysis

a. Samples and standards were transferred into g.l.c. vials with a Pasteur pipette. One standard was run after every ten samples in an automated chromatograph (Pye Unicom Series 304).

TABLE 3.4A Summary of ANOVAs for *in sacco* DM degradation of straw and lucerne (g/g) incubated in the rumen of steers offered a straw–lucerne diet.

Source of variation	d.f.	Mean squares (x 10^6)	
		Straw	Lucerne
Whole units			
Steer	2	5279.9*	2560.4
Period	1	4610.4*	0
Residual	2	109.3	168.9
Sub-units			
Time	6	177348.6***	100904.1***
Period x Time	6	463.8	687.6
Residual	24	384.9	642.7
Total	41	26621.9	15376.4

* $P<0.05$; *** $P<0.001$

TABLE 3.5A Summary of ANOVAs for rumen pH, NH_3-N (mg/l) and total VFA (mmol/l) in steers and wethers offered a straw-lucerne diet.

Source of variation	d.f.	Mean Squares		
		pH	NH_3-N[2]	VFA
Whole units				
Species	1	0.384	229.0	4378.3
Residual	4	0.123	32.2	1940.7
Sub-units				
Period	1	0.016	137.5**	328.2
Period x species	1	0.044	130.8**	22.8
Residual	4	0.051	5.0	56.1
Sub-sub-units				
Time	4	0.053	496.2***	294.3**
Time x species	4	0.016	84.8***	245.4**
Time x period	4	0.008	55.4*	162.1*
Time x species x period	4	0.014	43.5*	101.4
Residual	32[1]	0.011	14.9	60.1
Total	59[1]	0.031	66.0	302.6

1. Less 1 missing value for NH_3-N.
2. Analyzed as (mg/l) divided by ten.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

APPENDIX 4.1A
Determination of chromium (Cr)
Reagents

a. Acid mixture/potassium bromate solution: 250 ml conc. sulphuric acid, 380 ml conc. orthophosphoric acid, 20 ml 10 % manganese sulphate ($4H_2O$) solution, 300 ml 4.5 % potassium bromate solution, 350 ml distilled water.

b. Calcium chloride solution. Approx. 4000 ppm Ca.

c. Sodium silicate solution 0.28 %

Sample preparation

a. 3 g of the dried ground sample were weighed into a 45 ml silica basin and ashed at 650 °C for at least 5 h.

b. After ashing, 30 ml of the acid mixture/potassium bromate solution were added to the cooled sample. The basin was covered with a watch glass and placed on a hotplate at low setting (150 °C) until most of the water from the bromate solution had boiled off. The temperature was then gradually raised to 250 °C and heating continued until white fumes of sulphur trioxide were observed.

c. After removing from the hot plate, the solution in the basin was allowed to cool, diluted with water and transferred carefully with water washings to a 200-ml graduated flask. 25 mls of calcium chloride and 10 mls of sodium silicate were then added to the flask before making up to the mark with water. This solution was filtered and transferred to storing bottles before analysis by atomic absorption spectophotometry.

Atomic absorption spectrophotometry

a. The sample-solutions were run on a Gallenkamp SP 90 with the following operating conditions:

Lamp current	7 µA
Slit width	0.08 mm
Wavelength	360
Air	5 l/min
Acetylene	2200 cc/min
Burner height	0.5 cm

b. Chromium concentration was determined from a standard curve obtained with standard solutions containing 0, 2.5, 5, 10, 12.5, 15, 20 and 25 ppm of Cr.

TABLE 4.2A Summary of ANOVAs to test part-effects for *in sacco* degradation (g/g) of straw and lucerne in sheep offered untreated barley straw supplemented with 320 g lucerne pellets/kg dietary DM.

Source of variation	d.f.	Mean squares ($x10^6$)	
		DM	
		Straw	Lucerne
Whole units			
Part	1	3450	1044
Sheep (block)	2	3020	1572
Residual	2	1342	587
Sub-units			
Period	1	2409	117
Period x Part	1	0	1677
Residual	4	621	778
Sub-sub-units			
Time	4	420560	213524
Time x Part	4	1555***	1155
Time x Period	4	819*	1181
Time x Part x Period	4	814	116
Residual	32	445	740
Total	59	29259	5218

* $P<0.05$; *** $P<0.001$

Table 4.3A Summary of ANOVAs, for *in sacco* degradation (g/g) of straw and lucerne in sheep offered untreated barley straw supplemented with increasing levels of lucerne pellets.

Source of variation	d.f.	Mean squares ($x10^6$)			
		Straw		Lucerne	
		DM	NDF	DM	CP
Whole units					
Blocks	2	2862	3011	786	1218
Diet[1]	3	4394	4392	4263	3989
Residual	6	2217	4381	2464	6694
Sub-units					
Period	1	5805**	4696	932	1187
Period x Diet	3	837	774	267	1331
Residual	8	239	630	1117	2261
Sub-Sub-Units					
Time	4	814220***	1002674***	427771***	593827***
Time x Diet	12	109	701	1327	2451
Time x Period	4	858	1753	500	755
Time x Diet x Period	12[2]	382	804	436	723
Residual	64[3]	712	1079	1054	1312
Total	120	29229	36159	15853	22757

1. g lucerne DM/kg total dietary DM: for straw 0, 160, 330 and 480; for lucerne 160, 320, 480 and 1000.
2. Less missing values: 1 for straw DM and NDF and lucerne DM and 2 for lucerne CP.
3. Less missing values 3 for straw DM and NDF; 3 for lucerne DM and 4 for Lucerne CP.

** $P<0.01$; *** $P<0.001$.

TABLE 4.4A Summary of ANOVAs for daily OM intake and digestion kinetic parameters of straw (S) and lucerne (L) incubated in the rumen of sheep offered untreated barley straw supplemented with increasing levels of lucerne pellets.

Source of variation	d.f.	Mean squares										
		OMI, g/kg $LW^{0.75}$		Lag time, h			Potential degr. ($x10^3$)			Degradation rate[3], per h		
		S	Total	S		L	S		L	S		L
				Period			Period			Period		
				1	2	1+2	1	2	1+2	1	2	1+2
Block	2	7.6	20.8	0.87	0.77	0.27	5601	4869	76	0.39	0.08	0.37
Diet[1]	3	15.3	285.5*	0.22	0.20	1.86*	13196	333	172	0.10	0.04	0.35
Linear	1	18.7	777.8**	0.24	0.01	-	3739	484	-	0.07	0.10	-
Dev.	2	8.4	39.3	0.21	0.30	-	17924	354	-	0.11	0.02	-
Residual	6[2]	32.7	50.4	0.29	0.08	0.26	7577	2170	1317	0.27	0.04	1.52
Total	11	23.4	109.1	0.38	0.24	0.70	8867	2160	779	0.25	0.05	1.00

1. g lucerne DM/kg total dietary DM: for OMI and straw parameters: 0, 160, 320 and 480; for lucerne parameters 160, 320, 480 and 1000.
2. Missing value for lag time, potential degradability and degradation rate of straw in period 1.
3. Mean Squares x 10^3.

* $P<0.05$; ** $P<0.01$

TABLE 4.5A Summary of ANOVAs for rumen pH and NH_3-N (mg/l) in sheep offered either untreated straw or ammonia-treated straw, both supplemented with increasing levels of lucerne pellets.

Source of variation	Untreated straw			Ammonia-treated straw		
	d.f.[2]	Mean squares		d.f.[4]	Mean squares	
		pH	NH_3-N		pH	NH_3-N
Missing values		5	12		0	3
Whole units						
Block	2	0.075	45.0	2	0.111	29.5
Diet[1] (D)	3	0.190	121.1*	3	0.119	110.7
Linear	1	0.344	8.4	1	0.191	154.9
Quadratic	1	-	351.7**	-	-	-
Dev	1[3]	0.114	3.1	2	0.083	88.6
Residual	6	0.178	24.8	6	0.092	38.8
Sub-units						
Period (P)	1	0.352*	0.7	1	0.027	17.8
P x D	3	0.002	2.6	3	0.034	19.3
Dev. lin.	1	0.001	0.0	1	0.034	21.6
Dev. quad.	1	-	7.8	-	-	-
Dev.	1[3]	0.003	0.2	2	0.034	18.1
Residual	8	0.037	27.0	8	0.027	11.2
Sub-sub-units						
Sampling time	4	0.246***	824.4***	3	0.475*	1504.8***
T x D	12	0.057***	59.5***	9	0.020	21.0
Dev. lin	4	0.116**	115.3***	3	0.025	35.3*
Dev. quad	4	-	29.6*	-	-	-
Dev.	4[3]	0.027*	33.6*	6	0.018	13.8
T x P	4	0.037*	16.1	3	0.014	22.3
T x D x P	12	0.018	25.2*	9	0.012	10.2
Dev. dev. lin.	4	0.021	39.8*	3	0.006	9.4
Dev. dev.quad.	4	-	13.0	-	-	-
Dev.	3	0.017	22.8	6	0.015	10.6
Residual	64	0.013	11.1	48	0.010	11.0
Total	119	0.046	53.9	95	0.039	66.8

1. g lucerne/kg total dietary DM: 0, 160, 320, 480.
2. Less missing values: for pH: 1 from sub-units residual and 4 from sub-sub-units residual; for NH_3-N: 1 from sub-units residual and 11 from sub-sub-unit residual.
3. Includes d.f. from any quadratic component for pH only.
4. Less missing values: for NH_3-N: 3 from the sub-sub-units residual.
5. Mean squares for NH_3-N concentrations (mg/l) divided by 10.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 4.6A Summary of ANOVAs for total concentration of VFA (TOT) (mmol/l) and molar proportions of acetic (AC), propionic (PROP) and n-butyric acid (BUT), in the rumen of sheep offered either untreated or ammonia-treated barley straw, both supplemented with increasing levels of lucerne pellets.

Source of variation	Mean squares									
	Untreated straw					Ammonia-treated straw				
	d.f.	TOT	AC[2]	PROP[2]	BUT[2]	d.f.	TOT	AC[2]	PROP[2]	BUT[2]
Missing values		4	5	5	4		0	0	0	0
Whole units										
Block	2	224	9.9	6.9	0.37	2	1261	2.4	0.48	1.28
Diet[1]	3	5767**	3.8	2.1	6.42*	3	879	20.0	2.21	10.80***
Lin.	1	10604**	6.6	4.3	18.57**	1	440	57.6*	5.74	30.23***
Quad.	1	5913*	4.6	2.0	0.58	1	188	2.5	0.28	1.15
Dev.	1	785	0.2	0.01	0.11	1	2010	0.05	0.62	0.93
Residual	6	576	11.6	5.8	0.94	6	655	5.8	4.33	0.24
Sub-units										
Time	4	1010**	1.5**	1.8**	0.39*	3	1190	14.3***	6.78***	1.85***
Time x Diet	12	785***	1.8**	0.6**	0.38*	9	214	4.0*	0.69	0.44**
Dev. lin.	4	1463***	0.6	0.9**	0.41	3	379	3.8	0.44	0.81**
Dev. quad.	4	527***	3.8***	0.9**	0.61	3	161	5.9*	1.11	0.46**
Dev.[1]	4	365**	1.1*	0.05	0.12	3	101	2.2	0.53	0.05
Residual	32	74	0.3	0.16	0.14	24	173	1.7	0.37	0.13
Total	59	668	2.5	1.4	0.65	47	399	4.6	1.47	1.04

1. g lucerne/kg total dietary DM: 0, 160, 320 and 480.
2. Analyzed as molar proportions in mmol/100 mmol.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 4.7A Summary of ANOVAs for intake, ME concentration and *in vivo* digestibility of the diets and N-balance traits in sheep offered untreated barley straw supplemented with lucerne.

Source of variation	d.f.	Mean squares				
		OMI, g/kg $LW^{0.75}$ per day			ME	
					Daily intake	Dietary
		Straw	Total	Total dig.	MJ per sheep	MJ/kg DM
Block	3	120.2	161.0	54.1	2.6	3.5
Diet[1]	3	2.2	196.5[1]	72.6*	3.3*	3.0
Lin.	1	1.8	546.9**	200.7**	9.4**	8.8*
Dev.	2	2.4	21.2	8.5	0.3	0.1
Residual	9	35.6	45.9	12.0	0.6	1.0
Total	15	27.3	83.5	27.2	1.2	1.5
		Digestibility[2], g/g				
		DM	OM	GE	NDF	MADF
Block	3	10336	8648	6785	7849	4864
Diet[1]	3	10772*	8315	10373	307	3330
Lin.	1	31612**	23892*	30076*	0	8
Dev.	2	352	527	521	461	4991
Residual	9	2709	2461	3904	2602	5534
Total	15	4725	3925	5774	2028	4959
		g N per sheep per day				CP dig[2] g/g
		Intake	Faeces	Urine	Balance	
Block	3	6.2	0.10	0.51	2.4	2234
Diet[1]	3	26.6**	0.48*	2.61*	8.2**	8838
Lin.	1	76.5***	1.36**	7.59**	23.2***	23366*
Dev.	2	1.7	0.04	0.13	0.6	1575
Residual	9	1.9	0.10	0.39	0.6	2698
Total	15	8.1	0.19	0.95	2.5	3833

1. See text for details
2. Mean Squares x 10^6

* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

TABLE 4.8A Summary of ANOVAs for *in sacco* degradation (g/g) of straw and lucerne in sheep offered ammonia-treated barley straw supplemented with increasing levels of lucerne pellets.

Source of variation	Straw DM		Lucerne		
				MS ($x10^6$)	
	d.f.	MS ($x10^6$)	d.f.	DM	CP
Whole-units					
Block	2	33012	2	3469	7284
Diet[1]	3	8865	2	7554	9811
Residual	6	24592	4	9097	17416
Sub-units					
Period	1	1926	1	3294	15071**
Period x Diet	3	3675	2	876	5662
Residual	8	2395	6	667	1094
Sub-sub-units					
Time	4	1108403***	4	296402***	490210***
Time x Diet	12	2264	8	1762	4753
Time x Period	4	492	4	1254	2386
Time x Diet x Period	12	1581	8	738	1821
Residual	64	1468	48	1843	3258
Total	119	40739	89	15355	26025

1. g lucerne DM/kg total dietary DM: for straw 0, 160, 320, 480; for lucerne 160, 320, 480.

TABLE 4.9A Summary of ANOVAs for daily OM intake and digestion kinetics parameters of straw (S) and lucerne (L) incubated in the rumen of sheep offered ammonia-treated barley straw supplemented with increasing levels of lucerne pellets.

Source of variation	Mean squares									
	d.f.		OMI g/kg $LW^{0.75}$		Lag time, h		Potential degr.($x10^3$)		Degradation rate[4], per h	
	S[2]	L[3]	S	Total	S	L	S	L	S	L
Block	2	2	30.6	43.6	1.19	3.39	10474	580	0.05	1.55
Diet[1]	3	2	16.1	428.2**	0.30	2.81	17062	261	0.05	0.88
Linear	1	1	0.1	1234.8***	0.73	5.60	4987	248	0.10	1.32
Dev.	2	1	24.1	49.7	0.08	0.02	23099	274	0.02	0.44
Residual	6	4	24.0	25.3	1.10	3.02	17526	308	0.31	6.66
Total	11	8	23.1	138.5	0.90	3.05	16118	364	0.19	3.94

1. g lucerne DM/kg total dietary DM for OMI and straw parameters: 0, 160, 320, 480; for lucerne parameters: 160, 320, 480.
2. d.f. for OMI and straw parameters.
3. d.f. for lucerne parameters only.
4. Mean squares x 10^3.

** $P<0.01$; *** $P<0.001$

TABLE 4.10A Summary of ANOVAs for intake, ME concentration and *in vivo* digestibility of the diets and N-balance traits in sheep offered ammonia-treated barley straw supplemented with lucerne.

Source of variation	d.f.	Mean squares				
		OMI, g/kg $LW^{0.75}$ per day			ME	
					Daily intake	Dietary
		Straw	Total	Total dig.	MJ per sheep	MJ/kg DM
Block	3	46.9	63.5	17.1	1.1	0.15
Diet[1]	3	51.9	251.6*	51.5	2.9	0.69
Lin.	1	137.4	641.5**	122.4*	7.1**	2.00*
Quad.	1	12.3	95.4	25.5	1.2	0.07
Dev.	1	6.2	17.8	6.5	0.2	0.01
Residual	9	30.1	45.1	19.1	1.3	0.26
Total	15	35.5	96.7	27.2	1.7	0.37
		Digestibility[2], g/g				
		DM	OM	GE	NDF	MADF
Block	3	642	802	660	1208	1310
Diet[1]	3	894	1884	1087	17748***	11420**
Lin.	1	2648	5621*	3082	52817***	34067***
Quad.	1	13	10	156	324	32
Dev.	1	22	22	23	103	161
Residual	9	802	766	946	727	1159
Total	15	825	1046	917	4982	3241
		g N per sheep per day				CP dig[2] g/g
		Intake	Faeces	Urine	Balance	
Block	3	5.5	0.17	1.47	2.92	635
Diet[1]	3	69.1**	0.80*	16.98**	13.98**	3293*
Lin.	1	195.8***	2.41**	49.33***	29.36**	8153*
Quad.	1	10.9	0.00	0.17	8.50*	1726
Dev.	1	0.7	0.00	1.45	4.08	0
Residual	9	4.7	0.15	1.38	1.37	842
Total	15	20.8	0.31	5.28	4.52	1291

1. See text for details
2. Mean Squares x 10^{6}

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 5.1A Summary of ANOVAs to test the effect of sample size (5, 10 and 15 g DM) and sieving time (10, 15, 20 and 25 min) on the particle size distribution in rumen digesta from sheep offered straw-based diets.

Source of variation	d.f.	Mean squares of g DM retained per sieve (s)/kg DM retained on six sieves.						
		Sieve aperture size, mm						
		>1.18	4.75	2.36	1.18	0.60	0.30	0.15
Sample size	2	2031	4730	860*	7239***	7058**	553	437
Lin.	1	3521	8919*	1330*	13762***	14089***	902	860
Quad.	1	540	542	391	716*	27	205	14
Time	3	681	313	164	570*	550	2586	586
Lin.	1	1926	8	296	573*	859	3104	306
Quad.	1	115	923	195	1130**	78	3057	1277
Dev.	1	0	10	0	7	712	1595	174
Sample size x time	6	843	284	146	213	295	1384	1124
Lin. Lin.	1	22	119	131	17	349	974	297
Quad. Lin.	1	510	187	2	55	154	0	105
Lin. Quad.	1	2341	13	588	768*	14	1488	38
Quad. Quad.	1	1369	962	8	79	99	1790	233
Lin. Dev.	1	116	43	1	332	394	3740	932
Dev.	1	699	381	144	25	760	313	5140
Residual	12	1678	1287	221	98	718	1224	1864
Total	23	1361	1198	249	811	1137	1385	1380

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 5.2A Summary of ANOVAs to test the effect of method of sampling on the DM concentration and the particle size distribution in rumen digesta from sheep offered straw based diets.

Source of variation	d.f.	Mean squares[3]									
		DM g/kg	DM retained[2] g/kg	Sieve aperture size, mm							MF[4]
				4.75	2.36	1.18	0.60	0.30	0.15	>1.18	<1.18	
Diet[1] (D)	1	166	3169	59180	199	1076	4523	5661	14273	68621	68621	1.10
Method[1] (M)	1	631	19	4830	64	2175	170	1662	166	221	221	0.03
D x M	1	79	1564	176	2	2	18	1212	396	113	113	0.00
Residual	8	148	1324	5359	169	168	460	1182	1742	4697	4697	0.09
Total	11	187	1395	9733	147	418	763	1635	2616	9684	9684	0.17

1. Untreated or ammonia-treated straw. See text (Section 5.2.4.3) for methods of sampling of rumen digesta.
2. g/kg total sample DM.
3. Mean squares of DM retained per sieve/kg total DM retained on all sieves.
4. Modulus of fineness.

TABLE 5.3A Summary of ANOVAs for intake, faecal particle size distribution, rate of size reduction of large particles (k_r), rate of escape of small particles (k_{es}) and rate of escape of large and small particles (k_{els}) from the rumen of sheep offered straw-lucerne diets[1].

Source of variation	d.f.	Mean squares				Mean squares ($\times 10^{-5}$)		
		OM intake,		Faecal particles retained[3]		Fractional rates,		
		g/kg $LW^{0.75}$ per day		Sieve aperture size, mm		per h		
		Straw	Total	>1.18 mm	<1.18 mm	k_r	k_{es}	k_{els}
Diet[1]	3	204.4*	494.8**	2638*	2638*	14.4	11.7&	11.4
Residual	8[2]	35.2	44.4	431	431	24.4	3.10	4.9
Total	11[2]	81.3	167.3	1033	1033	21.6	5.7	6. 9

1. Untreated and ammonia-treated straw supplemented with 0 and 480 g lucerne DM per kg of total dietary DM.
2. One missing value for k_{es} and k_{els}.
3. g DM per sieve (s) per kg DM retained on six sieves (4.75, 2.36, 1.18, 0.6 and 0.15 mm aperture size).

& $P<0.1$; * $P<0.05$; ** $P<0.01$

TABLE 6.1A Summary of ANOVAs for OMI (g/kg $LW^{0.75}$ per day) by rumen-fistulated sheep offered either untreated or ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.	Mean squares			
		Untreated straw		Treated straw	
		Straw OMI	Total OMI	Straw OMI	Total OMI
Block	2	0.75	2.31	26.27	140.65
Diet[1]	3	13.93	24.47	4.64	15.25
Lin.	1	0.77	1.13	11.91	39.09
Quad.	1	7.06	11.10	0.00	1.22
Dev.	1	33.98	61.18	2.02	5.44
Residual	6[2]	23.11	43.35	22.37	82.19
Total	11[2]	16.54	30.74	17.83	73.80

1. See text for details
2. One missing value for treated-straw diets.

TABLE 6.2A Summary of the ANOVA for *in sacco* degradation (g/g) of straw DM in the rumen of sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.	Mean squares (x 10^6)
Whole units		
Block	2	14645*
Diet[1]	3	3436
Residual	6	1978
Sub-units		
Period[1]	1	21323**
Period x Diet	3	133
Residual	8	938
Sub-sub-units		
Time[1]	4	721258***
Time x Diet	12	701
Time x Period	4	1064
Time x Diet x Period	12	605
Residual	64	439
Total	119	253255

1. See text for details
* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 6.3A Summary of ANOVAs for rumen pH and NH_3-N concentration (mg/l) in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.[2]	Mean squares	
		pH[3]	NH_3-N
Missing values		4	4
Whole units			
Block	2	4.5	6204
Diet[1] (D)	3	70.1	3816
Linear	1	4.0	2095
Quad.	1	205.7	6203
Dev.	1	0.5	3149
Residual	6	348.9	7872
Sub-units			
Period[1] (P)	1	0.3	187
P x D	3	9.4	170
Dev. lin.	1	18.9	90
Dev. quad.	1	6.7	31
Dev.	1	2.5	388
Residual	8	46.0	934
Sub-sub-units			
Sampling time[1] (T)	3	990.5***	125945***
T x D	9	10.4	1609
Dev. lin.	3	11.2	2359
Dev. quad.	3	11.3	1531
Dev.	3	8.6	939
T x P	3	14.3	852
T x D x P	9	12.1	200
Dev. dev. lin.	3	10.3	440
Dev. dev. quad.	3	23.2	131
Dev.	3	2.9	29
Residual	48	13.4	1062
Total	95	71.2	5745

1. See test for details.
2. Without substracting missing values.
3. pH mean squares x 10^3.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 6.4A Summary of ANOVAs for total concentration of VFA (TOT) (mmol/l) and molar porportions of acetic (AC), propionic (PROP) and n-butyric acid (BUT) in the rumen of sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.[2]	Mean squares			
		TOT	AC[3]	PROP[3]	BUT[3]
Missing values		2	2	2	2
Whole units					
Block	2	48	13.8	8.0	1.2
Diet[1] (D)	3	1271	8.6	3.9	3.0
Lin.	1	2	4.0	8.1	0.1
Quad.	1	36	4.5	0.5	0.8
Dev.	1	3777*	17.4	3.1	8.0
Residual	6	292	5.3	5.5	1.9
Sub-units					
Sampling time[1] (T)	3	822***	14.3***	1.9*	0.9**
T x D	9	127	0.7	0.3	0.3
Dev. lin.	3	131	0.5	0.2	0.8
Dev. quad.	3	6	0.3	0.1	0.0
Dev.	3	245	1.3	0.7	0.1
Residual	24	72	1.1	0.5	0.2
Total	47	241	3.5	1.8	0.7

1. See text for details
2. Without substracting missing values.
3. Mean squares x 10^4.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 6.5A Summary of ANOVAs for intake, ME concentration and *in vivo* digestibility of the diets and N-balance traits in sheep offered untreated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.	Mean squares				
		OMI, g/kg $LW^{0.75}$ per day			ME	
					Daily intake	Dietary
		Straw	Total	Total dig.	MJ per sheep	MJ/kg DM
Block	3	24.5	50.0	13.4	0.89	0.04
Diet[1]	3	29.4	58.8	58.4*	2.20*	1.37
Lin.	1	64.3*	129.5*	138.7**	5.47**	3.26*
Quad.	1	23.9	46.5	29.4	1.13	0.78
Dev.	1	0.0	0.5	7.0	0.00	0.06
Residual	9	11.5	22.0	10.7	0.49	0.57
Total	15	17.7	35.0	20.8	0.92	0.62
		Digestibility[2], g/g				
		DM	OM	GE	NDF	MADF
Block	3	6.0	6.8	2.9	8.1	7.7
Diet[1]	3	69.1*	81.7*	62.2	72.8*	69.7*
Lin.	1	157.2**	197.4**	136.9*	150.7**	164.5**
Quad.	1	16.8	20.4	39.1	29.0	18.6
Dev.	1	33.3	27.3	10.6	38.8	26.0
Residual	9	13.2	12.8	17.4	13.4	15.9
Total	15	22.9	25.4	23.4	24.2	25.0
		g N per sheep per day				CP digesti-bility[2], g/g
		Intake	Faeces	Urine	Balance	
Block	3	3.83	0.15	1.96	0.21	2.5
Diet[1]	3	4.06*	0.04	1.28*	1.07	27.1*
Lin.	1	10.47**	0.02	2.52*	3.18*	67.6**
Quad.	1	1.69	0.02	1.01	0.02	1.2
Dev.	1	0.01	0.09	0.30	0.02	12.5
Residual	9	0.77	0.05	0.27	0.44	4.5
Total	15	2.04	0.06	0.81	0.52	8.6

1. See text for details
2. Mean Squares x 10^4

* $P<0.05$; ** $P<0.01$

TABLE 6.6A Summary of ANOVAs for *in sacco* degradation (g/g) of straw DM in the rumen of sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.	Mean squares (x 10^6)
Whole units		
Block	2	7778
Diet[1]	3	24677*
Lin.	1	30149*
Quad.	1	27649*
Dev.	1	16234
Residual	6	4017
Sub-units		
Period[1]	1	19789***
Period x Diet	3	1484
Dev. lin.	1	0
Dev. quad.	1	998
Dev.	1	3453
Residual	8	739
Sub-sub-units		
Time[1]	4	1096106***
Time x Diet	12	3388***
Dev. lin.	4	4344***
Dev. quad.	4	3322**
Dev.	4	2499**
Time x Period	4	1155
Time x Diet x Period	12	633
Lin. dev. dev.	4	637
Quad. dev. dev.	4	943
Dev.	4	320
Residual	64	628
Total	119	374969

1. See text for details.
* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 6.7A Summary of ANOVAs for rumen pH and NH_3-N concentration (mg/l) in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.[2]	Mean squares	
		pH[3]	NH_3-N
Missing values		0	1
Whole units			
Block	2	106.7	884
Diet[1] (D)	3	369.3	19240*
Linear	1	561.2	26886*
Quad.	1	268.8	19114
Dev.	1	277.9	11719
Residual	6	221.5	4076
Sub-units			
Period[1] (P)	1	6.0	339
P x D	3	86.8	1879
Dev. lin.	1	0.8	5545
Dev. quad.	1	117.6	59
Dev.	1	142.1	33
Residual	8	62.5	2253
Sub-sub-units			
Sampling time[1] (T)	3	876.8***	148863***
T x D	9	44.8*	4694***
Dev. lin.	3	57.3*	7343***
Dev. quad.	3	35.3	6165***
Dev.	3	41.9*	572
T x P	3	11.8	5268***
T x D x P	9	7.6	175
Dev. dev. lin.	3	0.6	144
Dev. dev. quad.	3	13.1	101
Dev.	3	9.0	279
Residual	48	19.6	779
Total	95	78.9	6923

1. See text for details.
2. Without substracting missing values.
3. pH mean squares x 10^3.

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 6.8A Summary of ANOVAs for total concentration of VFA (TOT) (mmol/l) and molar porportions of acetic (AC), propionic (PROP) and n-butyric acid (BUT) in the rumen of sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.	Mean squares			
		TOT	AC[2]	PROP[2]	BUT[2]
Whole units					
Block	2	284	1.2	1.7	0.86
Diet[1] (D)	3	1712	12.3	6.1*	5.61
Lin.	1	3354$^{\&}$	24.1*	0.1	16.40*
Quad.	1	1322	0.1	1.0	0.01
Dev.	1	460	12.7	17.2**	0.44
Residual	6	697	3.2	1.0	2.19
Sub-units					
Sampling time[1] (T)	3	1406*	31.7***	0.2	4.33***
T x D	9	779	1.5	0.1	0.91**
Dev. lin.	3	2077**	2.7	0.1	2.25***
Dev. quad.	3	91	1.6	0.0	0.42
Dev.	3	167	0.2	0.1	0.05
Residual	24	402	0.6	0.2	0.27
Total	47	654	3.9	0.7	1.26

1. See text for details
2. Mean squares x 10^4.
& $P<0.1$; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 6.9A Summary of ANOVAs for intake, ME concentration and *in vivo* digestibility of the diets and N-balance traits in sheep offered ammonia-treated barley straw supplemented with lucerne and sugar beet pulp.

Source of variation	d.f.	Mean squares				
		OMI, g/kg $LW^{0.75}$ per day			ME	
					Daily intake	Dietary
		Straw	Total	Total dig.	MJ per sheep	MJ/kg DM
Block	3	20.0	54.0	27.4	2.02	0.08
Diet[1]	3	5.7	16.6	42.9*	2.49*	3.15***
Lin.	1	5.1	3.3	94.6**	6.48**	8.90***
Quad.	1	11.4	41.9	14.5	0.31	0.00
Dev.	1	0.7	4.5	19.5	0.68	0.54*
Residual	9	9.9	29.8	8.2	0.49	0.09
Total	15	11.1	32.0	19.0	1.19	0.70
		Digestibility[2], g/g				
		DM	OM	GE	NDF	MADF
Block	3	1.9	1.8	2.3	1.5	2.2
Diet[1]	3	100.0***	129.0***	124.6***	144.0***	107.6***
Lin.	1	282.2***	363.9***	346.5***	406.7***	284.6***
Quad.	1	0.6	0.1	0.7	0.0	0.4
Dev.	1	17.4*	23.1*	26.7*	25.1*	37.7*
Residual	9	2.1	2.9	2.9	4.3	4.7
Total	15	21.6	27.9	27.1	31.7	24.8
		g N per sheep per day				CP digestibility[2], g/g
		Intake	Faeces	Urine	Balance	
Block	3	8.49	0.66	3.35	14.93	11.0
Diet[1]	3	1.83	0.97*	0.59	1.03	23.8*
Lin.	1	3.63	1.85**	0.36	0.00	35.0*
Quad.	1	1.81	0.05	0.73	0.51	5.9
Dev.	1	0.05	1.01*	0.68	2.60	30.4*
Residual	9	3.16	0.15	2.39	4.72	3.6
Total	15	3.96	0.42	2.22	6.02	9.1

1. See text for details
2. Mean Squares x 10^4

* $P<0.05$; ** $P<0.01$; *** $P<0.001$

TABLE 6.10A Summary of ANOVAs for ME concentration and *in vivo* digestibility of the diets and N-balance traits in sheep fed pellets containing various proportions of lucerne and sugar beet pulp.

Source of variation	d.f.	Mean squares			
		ME		Digestibility[2], g/g	
		Daily intake MJ per sheep	Dietary MJ/kg DM	DM	OM
Diet[1]	2	1.7	5.8 ***	181.4***	278.5 ***
Lin.	1	2.1	11.7 ***	362.2***	553.9 ***
Dev.	1	1.4	0.01	0.6	3.2
Residual	9	0.4	0.09	3.7	2.6
Total	11	0.6	1.14	36.0	52.7

		Digestibility[2], g/g			
		GE	NDF	MADF	CP
Diet[1]	2	288.6***	696.4***	682.4***	0.07
Lin.	1	574.6***	1392.7 ***	1360.7***	0.01
Dev.	1	2.5	0.0	4.1	0.14
Residual	9	3.4	6.2	14.9	2.2
Total	11	55.2	131.7	136.3	1.8

		g N per sheep per day			
		Intake	Faeces	Urine	Balance
Diet[1]	2	61.5***	1.9 ***	27.9***	1.6
Lin.	1	122.4***	3.7 ***	56.0***	2.7
Dev.	1	0.6	0.03	0.01	0.5
Residual	9	0.9	0.03	0.17	0.6
Total	11	11.9	0.37	5.2	0.8

1. See text for details.
2. Mean squares x 10^4.
*** $P<0.001$