WHEAT STRAW AS RUMINANT FEED

EFFECT OF SUPPLEMENTATION AND AMMONIA TREATMENT ON VOLUNTARY INTAKE AND NUTRIENT AVAILABILITY

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Effect of Supplementation and Ammonia Treatment on Voluntary Intake and Nutrient Availability

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Abstract
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This thesis describes the results of experiments with goats, sheep and cattle fed untreated or ammonia-treated wheat straw. Aim of the experiments was to identify factors limiting voluntary intake and digestion of these low-quality feeds. Supplementation of urea to untreated wheat straw increased *in vitro* degradation if the ammonia-nitrogen concentration in the substrate was below 60-100 mg/l. No effect of urea supplementation to untreated wheat straw on digestion was observed in sheep and goats. Voluntary straw intake increased, however, in goats, but not in sheep when urea was supplemented to untreated wheat straw. Ammonia treatment increased intake and digestion in sheep as well as in cattle. The increased rumen turnover as a result of ammonia treatment was associated with an increased potentially degradable fraction of the ingested straw. The fractional rate of passage, estimated from the faecal excretion pattern of Cr-NDF and the fractional rate of degradation derived from *in sacco* studies were not much affected by ammonia treatment. Validation of these rate constants of passage and degradation by model estimates derived from a rumen evacuation study showed that the *in sacco* rate of degradation underestimated and that the rate of passage derived from the faecal excretion pattern of Cr-NDF overestimated the actual values. Digestible energy intake from untreated and ammonia-treated wheat straw by sheep and cattle were equalized by scaling to liveweight$^{0.946}$. There were strong indications that maintenance requirements accross sheep and cattle were related to liveweight to an exponent lower than 0.9. Cattle were therefore more efficient with regard to utilization of rations based on untreated and ammonia-treated wheat straw than sheep. Although ammonia treatment resulted in a considerably increased digestible energy intake, the availability of amino acids for absorption from the small intestine remained relatively low as a consequence of a low efficiency of rumen microbial protein synthesis and a low duodenal flow of rumen undegraded dietary amino acids. Supplementation of casein, a protein of a high rumen degradability or of potato protein of a relatively low rumen degradability, to ammonia-treated wheat straw increased the efficiency of microbial protein synthesis, whereas potato protein also increased the duodenal flow of undegraded dietary amino acids. Casein supplementation resulted in substitution of straw by the supplement intake, whereas digestible energy intake increased as a result of potato protein supplementation. It was concluded that voluntary digestible energy intake of rations based on wheat straw was primarily limited by the duodenal availability of protein and not by the rumen processing capacity.

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Voorwoord

Het voor u liggende proefschrift beschrijft een aantal experimenten uitgevoerd bij de vakgroepen Veehouderij (sectie Tropische Veehouderij) en Fysiologie van Mens en Dier van de Landbouwuniversiteit. Ik ben deze vakgroepen zeer erkentelijk voor de ruimte en mogelijkheid die mij geboden is om dit proefschrift tot stand te doen komen.

De behandeling van stro met ammoniak bleek in de praktijk moeilijker dan ik gedacht had. Zonder de assistentie van Ton Roos van de vakgroep Fysiologie van Mens en Dier, de medewerking van het Buro Veiligheid en Milieuhygiëne en de hulp van de medewerkers van de proefaccomodatie "De Ossekampen" was het stro altijd onbehandeld gebleven. Ik ben jullie dan ook zeer erkentelijk.

De experimenten hebben veel beslag gelegd op zowel de proefaccomodatie als de laboratoria bij de vakgroep Fysiologie van Mens en Dier en ik wil de volgende medewerkers van deze vakgroep dan ook met name bedanken:

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Voorts is een aanzienlijk deel van de praktische kant van de experimenten gedragen door medewerkers van de vakgroep Veehouderij, sectie Tropische Veehouderij. Ik wil dan ook met name Anne Waanders voor de assistentie bij de uitvoering van de proeven en Rein Ketelaar en Inge van Langenvelde voor de chemische analyses bedanken. Ik heb de samenwerking met jullie als uiterst prettig ervaren.

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Ikzelf ben blij, dat de klus geklaard is en het leven langzaam, maar zeker weer normaal wordt. Ik weet zeker, dat Karin, Roos en Tjerk zich met mij verheugen.

STELLINGEN

1. De vrijwillige opname van verteerbare organische stof uit laagwaardige ruwvoeders door herkauwers wordt *niet* primair beperkt door de verwerkingscapaciteit van het pens-netmaag-complex.

   Dit proefschrift.

   Ketelaars, J.J.M.H. and B.J. Tolkamp, 1992. Toward a new theory of feed intake regulation in ruminants 1. Causes of differences in voluntary feed intake; critique of current views. Livestock Production Science 30: 269-296.

2. De fractionele verteringssnelheid van potentiëel verteerbaar voer in de pens wordt onderschat met de *in sacco* methodiek en de fractionele verdwijningssnelheid van kleine deeltjes uit de pens wordt overschat wanneer deze gebaseerd is op het uitscheidingspatroon van merkstoffen in de faeces.

   Dit proefschrift.

   Aitchisson, E., M. Gill, J. France and M.S. Dhanoa, 1986. Comparison of methods to describe the kinetics of digestion and passage of fibre in sheep. Journal of the Science of Food and Agriculture 37: 1065-1072.

3. De bijdrage van microbiële fermentatie in het blinde darm/dikke darm-complex van herkauwers aan de totale celwandvertering is gering.

   Dit proefschrift.

4. Bij *ad libitum* gevoerde rantsoenen lijkt de kwaliteit van het rantsoen geen effect te hebben op de efficiëntie van benutting van zowel metaboliseerbare energie als van werkelijk uit de dunne darm geresorbeerd eiwit.

   Dit proefschrift.

   Tolkamp, B.J. and J.J.M.H. Ketelaars, 1993. The effect of ad libitum feeding on the efficiency of energy utilization in growing and lactating cattle. Animal Production 56: 431-432.

5. Onder omstandigheden waarin toevoeging van een energiesupplement of onbestendig eiwit aan een rantsoen gebaseerd op laagwaardige ruwvoeders resulteert in volledige substitutie van de verteerbare organische stof opname van het ruwvoeder door die van het supplement kan toevoeging van bestendig eiwit de totale opname van verteerbare organische stof verhogen.

   Dit proefschrift.

6. Pensmodellen verschaffen geen inzicht in causale verbanden tussen voeropname en verdwijning van materie uit de pens.

7. De grootste uitdaging voor fundamenteel onderzoek op het gebied van de voeding van één- en meermagigen is de regulering van de voedselopname.

8. Bij de veredeling van graangewassen voor teelt in tropische gebieden zouden zowel de kwaliteit als de kwantiteit van het stro onderdeel van de selectiecriteria moeten zijn.

9. Het feit, dat werknemers in de gezondheidszorg een hoog ziekteverzuim hebben is tekenend voor de arbeidsomstandigheden in deze sector.

10. De marginalisatie van tropische richtingen aan de Landbouwuniversiteit door fusies en stille liquidaties is in strijd met haar op internationalisering gerichte beleid.

11. De duurzaamheid van ontwikkelingsprojecten is dikwijls groter dan die van de beleidsdoelstellingen van het Ministerie van Ontwikkelingssamenwerking die aan deze projecten ten grondslag liggen.

12. Overtreding van het aanlijngebod voor honden is eerder regel dan uitzondering.

13. Vergelijking van de speelsterkte van damcomputers met die van menselijke dammers is even zinloos als elke andere vergelijking tussen mensen en machines.

Stellingen behorend bij het proefschrift: Wheat straw as ruminant feed: effect of supplementation and ammonia treatment on voluntary intake and nutrient availability, Simon J. Oosting, 10 november 1993.

Contents

CHAPTER 1.
GENERAL INTRODUCTION

UTILIZATION OF STRAW FOR RUMINANT FEEDING

1. Utilization of straw for ruminant feeding

During the last decade the number of publications about utilization of straw for animal feeding and particularly about upgrading of the nutritive quality of straw through alkaline treatment has been enormous (e.g. Sundstøl and Owen, 1984; Doyle et al., 1986; Singh and Schiere, 1988; Chenost and Reiniger, 1989; Schiere and Ibrahim, 1989; Ibrahim et al., 1992). The attention for straw as animal feed is based on the fact that this fibrous crop-residue comprises the main basal feed for ruminants in large parts of the world, especially in South East Asia. The growing human population in this region increased pressure on land for crop production and consequently reduced the area available for forage production and for rangelands. Livestock production systems became therefore more and more dependent on by-products of food production for human consumption. The growing human population not only reduced the area of land available for livestock production, but also increased the demand for livestock products as milk and meat for food and dung for fuel and fertilization. In addition, in the growing economies in South East Asia, where the average per capita income increases at a relatively high rate (World Bank, 1991), the demand for meat and milk also increases at a high rate. Many countries in South East Asia face therefore the need either to increase livestock production from a diminishing area or to import livestock products. The latter may be economically feasible, but is often disadvantageous, not only with regard to expense of (often scarce) foreign exchange and dependency on the world market with fluctuating prices, but also because importation may have a negative effect on local production and consequently on employment possibilities in rural areas. Maximum utilization of available resources to increase local production from ruminant livestock is therefore often an alternative preferable to importation.

Availability of crop-residues

Kossila (1988) estimated the availability of fibrous crop-residues per ruminant livestock unit of 500 kg. In South-East Asia availability of fibrous crop-residues in 1981 ranged from approximately 2-4 (India) to more than 10 metric tonnes (Indonesia) per ruminant livestock unit of 500 kg per annum (see Figure 1). In Africa and South America availability of fibrous crop-residues was generally lower, ranging from 0-4 metric tonnes per ruminant livestock unit per annum. In these regions large areas of arid to semi-arid rangelands are found and livestock production is therefore only to a limited extent dependent on fibrous crop-residues.

Daily dry matter intake from fibrous crop-residues usually does not exceed 2 kg/100 kg liveweight (Doyle et al., 1986). Dry matter requirements from fibrous crop-residues for a livestock unit of 500 kg are therefore approximately 3.6 metric tonnes per year. This indicates that the availability of fibrous crop-residues in 1981 was approximately sufficient to feed the ruminant livestock population in India and exceeded the requirements in many other South East Asian countries.

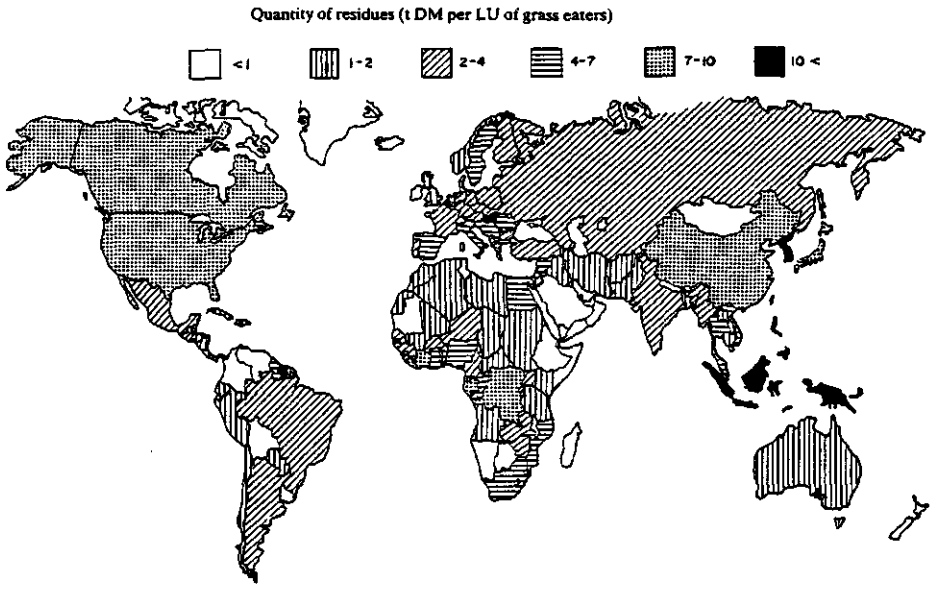

Figure 1. Production of fibrous crop residues in different countries of the world in 1981, tonnes dry matter per ruminant livestock unit of 500 kg per year. (Source: Kossila, 1988)

However, availability of fibrous crop-residues may differ between regions within a country and may be low in years or seasons with a below average rainfall. In addition, alternative purposes of utilization of crop-residues, e.g. as resource for the paper industry, as thatching material for roofs, as fuel and as fertilizer may reduce the availability for ruminant feeding.

Breeding of cereal cultivars during the last decades has been aimed primarily at increasing grain yield. Straw yields of improved varieties are often much lower than those of traditional varieties. Comparison of traditional and improved varieties (cultivated under identical conditions) of spring barley (Riggs et al., 1981), winter wheat (Austin et al., 1980) and finger millet (Gowda et al, 1988) showed that straw yields of the respective crops were 26, 16 and 35 % lower for improved than for traditional varieties. Increasing introduction of improved varieties may result eventually in reduced straw availability.

Kossila (1988) concluded that during the period 1970-1981 global availability of

fibrous crop-residues increased at a higher rate than did livestock numbers. Estimates of availability of crop-residues were derived from cereal production data and converted to fibrous by-product quantities by a multiplier. This multiplier, the straw to grain ratio, was kept constant for each cereal species over the period 1970-1981. The incremental rate of crop-residue availability would have been lower if a correction was made for the decrease in straw/grain ratio for the various cereal species, which probably occurred during this period. In India, decreasing availability of sorghum straw in relation to requirements resulted in increasing straw prices relative to grain prices during the period 1970-1991 (Kelley et al., 1991). These authors therefore concluded that straw yields should not be neglected as a factor for cereal breeding programs in areas where farmers depend to a large extent on straw for ruminant feeding.

Technologies aimed at increasing the nutritive value of straw often result in increased voluntary straw intake. For example, effects of ammonia treatment of straws on energy intake are partly attributed to an increased voluntary intake in addition to an increased energy digestion (Doyle et al., 1986; Dias-da-Silva and Sundstøl, 1986; Silva et al., 1989). Limited availability of fibrous crop-residues could restrict the quantity offered by a farmer, thus limiting the beneficial effects of alkaline treatment and/or supplementation.

Livestock productivity per unit land is obviously more determined by availability of high-quality roughages and concentrates than by the availability of fibrous crop-residues. Availability of concentrates is often limited in tropical countries and part of the locally available agro-industrial by-products, like groundnutcakes and coconutcakes, are often exported in order to get foreign exchange. Under such conditions national livestock production could be increased by using all the available high-quality feeds for a relatively small number of animals with high genetic potential (Zemmelink, 1986). Part of the available low-quality feeds would then remain unused and a considerable reduction of the animal number would be inevitable. Ruminant livestock, however, is required for draught purposes, production of low quantities of milk and/or meat for infants, dung production for fuel and fertilization and for economic safety of poor rural families. Though maybe not economically most feasible, livestock production from a large number of animals with a low, but optimal production under the given availability of resources, is socially more acceptable. Under these circumstances, methods to increase the nutritive quality of straw could either reduce the requirements for better quality feeds to achieve a desired production level or increase the ruminant livestock productivity per unit land.

Nutritive quality of straw.

Straws are characterized by a high fibre and low protein content and an unbalanced mineral composition (O'Donovan, 1983, Sundstøl and Owen, 1984, Doyle et al., 1986). Voluntary intake and digestion of straws are generally low. Digestion is low, because rumen

microbes degrade the straw's fibre at a relatively slow rate and only to a limited extent. This is partly an intrinsic characteristic of the straw's fibre, but is also a result of the unbalanced nutrient availability for microbial degradation in the rumen. Microbes require nitrogen, true protein, energy and minerals and each of these nutrients may limit microbial degradation of low-quality feeds (Hespell and Bryant, 1979; Hoover, 1986; Silva et al., 1989).

The mechanism regulating voluntary intake of low-quality feeds is still far from sufficiently understood. Proposed mechanisms range from almost entirely physical to almost entirely physiological.

The commonly accepted theory of feed intake regulation, the so-called physical intake regulation mechanism, states the rumen processing capacity as the major determinant of voluntary feed intake (e.g. Conrad, 1966; Baile and Forbes, 1974). The rumen processing capacity is determined by rumen fill, the rate of degradation of potential degradable matter in the rumen, the rate at which large rumen particles, unable to leave the rumen, are comminuted to small particles by rumination and the rate at which small rumen particles leave the rumen (Bosch et al., 1992). Ruminants fed low-quality feeds are unable to achieve the required intake level to meet the genetically determined demand for nutrients for milk or meat production or even maintenance, because voluntary intake is restricted by the rumen fill and processing capacity.

Part of the processing in the rumen is determined by intrinsic characteristics of the feed ingested, like rate and potential extent of degradation. Other factors associated with rumen processing capacity, like tolerated level of rumen fill and rate of passage of undegraded matter from the rumen, are, however, not only determined by feed factors, but can be manipulated by the animal. There is evidence from literature that environment, physiological status of the animal and nutrient availability may have an effect on rumen processing capacity without effect on rumen degradation. A decrease of temperature below the lower critical level or shearing in sheep increased intake in association with an increased rumen volume and/or rate of passage from the rumen (Weston, 1982; Kennedy et al., 1986). Lactation also increases intake of a feed in association with an increased rumen volume and/or increased passage from the rumen (Agricultural Research Council, 1980; Van Soest, 1982; Weston, 1982; Adenuga et al., 1990). Increased small intestinal protein availability in sheep fed roughages increased the rumen turnover without effect on disappearance of dry matter from the rumen through degradation (Egan and Doyle, 1985; Doyle and Panday, 1990).

The fact that ruminants fed roughages are able to increase their rumen volume or passage rates from the rumen under certain conditions means that rumen processing capacity *per se* is not the major determinant of voluntary feed intake, but that the amount of feed that is processed in the rumen is governed by physiological mechanisms.

An alternative hypothesis of regulation of voluntary feed intake was given by Tolkamp and Ketelaars (1992). They proposed as the primary physiological basis of all animal behaviour, including feed intake, that animals tend to minimize costs relative to benefits of an activity. The cost of feed intake is oxygen consumption which, owing to the toxic effect

of oxygen radicals on tissue components, results in ageing and reduced vitality and longevity. Ruminants therefore minimize oxygen consumption per unit net energy consumed (the benefit of feed intake). The low intake of low-quality feeds relative to feeds of higher quality is explained by the lower efficiency of utilization of metabolizable energy *i.e.* the higher energy expenditure and the associated higher oxygen consumption for chewing, digestion and metabolization processes (Agricultural Research Council, 1980). The hypothesis that voluntary intake of low-quality feeds is limited by availability of protein to the tissues as proposed by Egan (1977), Egan and Doyle (1985), Preston and Leng (1987) and Doyle and Panday (1990) could probably be seen in line with the hypothesis of Tolkamp and Ketelaars (1992). The link between these two hypotheses of feed intake regulation is provided by the observation that increased protein availability may increase the efficiency of metabolizable energy utilization (MacRae and Lobley, 1982; MacRae et al., 1985), thus lowering the oxygen consumption per unit net energy ingested. Why metabolizable energy is not utilized to the maximum extent under protein limitation remains to be investigated. It could be that excess energy is oxidized completely and that the concomitant ATP production is lost in futile cycles, or, that excess energy *i.e.* a high volatile fatty acid load for cells, requires a high energy expenditure to maintain the cell homoeostasis (Ketelaars and Tolkamp, 1991). It is likely that limited availability of other nutrients, like precursors for gluconeogenesis and minerals, relative to energy availability to the tissues could limit the efficiency of metabolizable energy utilization and consequently voluntary energy consumption.

Alkaline treatment and supplementation of nutrients, limiting either microbial degradation in the rumen or utilization of other nutrients by the animal, are practically applied means to increase voluntary intake and digestion of straws. Other potential methods to upgrade the nutritive value of straws, but to a lesser extent practically applied, are: physical treatments (soaking and wetting, grinding, pelleting, gamma irradiation: Sundstøl and Owen, 1984; Doyle et al., 1986; steam treatment: Rangnekar et al., 1986), breeding and selection of varieties with a relatively high nutritive value (Capper, 1988; Ibrahim et al., 1992), other chemical treatments (e.g. oxidative and acid reagents: Doyle et al., 1986; Willms et al., 1991), management practices (harvest, storage: Doyle et al., 1986) and biological treatment (Flegel, 1988). As with alkaline treatment and supplementation, application of these alternative methods of straw improvement depends on technical and socio-economic feasibility within farming systems.

Alkaline treatment and supplementation

As described by Sundstøl and Owen (1984), it was found already at the end of the last century that boiling of straws with alkali increased the feeding value. Research aimed at application of alkaline treatment in the tropics started in the middle of the nineteen seventies (Jackson, 1977) and still continues at many places. The principle of alkaline treatment is that

the ester linkages between hemicellulose and lignin in cell walls of monocotyledons are disrupted by alkali, increasing primarily the availability of hemicellulose for degradation by rumen microbes (Morrisson, 1983; Chesson et al., 1993; Mason et al., 1990). Microbial degradation of cellulose may also increase, probably due to an enhanced microbial activity (Chesson et al., 1983). Differences in effects of various alkaline reagents (NaOH, $Ca(OH)_2$, KOH, ammonia and urea, which is converted to ammonia by urease activity during ensiling) were found, mainly as an effect of the pH at which the treatment occurs (Sundstøl and Owen, 1984).

Alkali-treated straw-based rations may require supplementation to balance nutrients for rumen microbes. Urea supplementation to NaOH-treated barley straw increased dry matter intake and digestion (Ørskov and Grubb, 1978), indicating that rumen availability of nitrogen was limiting an optimal utilization of the NaOH-treated barley straw. Treatment with ammonia or urea increases concomitantly the nitrogen availability in the rumen, but microbial activity in the rumen may be limited by sulphur availability required for synthesis of the sulphur containing amino acids methionine and cystine (Siebert and Kennedy, 1972). Rumen availability of phosphorus could also limit growth or fermentative activity of rumen microbes (Tamminga, 1986).

When urea is supplemented or when the straw is treated with ammonia or urea, a source of readily available energy, like molasses or sugar beet pulp, may be required to ensure efficient utilization of the readily available nitrogen (Doyle et al., 1986; Silva et al., 1989). Also true protein availability could limit microbial processes in the rumen. True protein acts as a source of oligo-peptides, branched chain volatile fatty acids and minerals (Hoover, 1986; Hespell and Bryant, 1989).

Availability of true protein as aminogenic and glucogenic nutrient in the lower gut could limit the animal's energy intake as a result of an imbalanced nutrient supply for metabolism (Egan, 1977; Preston and Leng, 1987). Intestinal protein availibility from straw based rations is presumed to be limited as a result of a low extent and efficiency of microbial protein synthesis in the rumen (Hespell and Bryant, 1979) and a low small intestinal availability of undegraded dietary protein (Hvelplund, 1989). In addition, small intestinally absorbed amino acids may be required for gluconeogenesis, because the fermentative production of propionate, which is a precursor for gluconeogenesis, is relatively low on low-quality feeds (Preston and Leng, 1987). Therefore, supplementation of nutrients to straw-based diets to improve the small intestinal availability of protein either by an increased rumen microbial protein synthesis or by an increased undegraded dietary protein flow from the rumen could increase, potentially, the intake of energy by ruminants fed straw-based rations.

Low supplementation levels may be applied to balance the nutrient availability to rumen microbes or to the host animals and may result in increased intake or digestion of the basal component of the ration. However, high supplementation levels, especially of energy, will result in substitution of straw by supplement intake. The substitution rate (decrease in straw intake/kg supplement) was higher for ammonia-treated rice straw than for untreated rice straw fed to cattle for supplement levels varying from 1 to 7 kg concentrate dry matter

per day (Doyle et al., 1986). However, protein supplementation up to levels of 30 % of total dietary dry matter intake had no negative effects on rice straw intake, probably as a result of the increased protein availability in the lower gut (Robinson and Stewart, 1968).

Scope of the thesis

The aim of the experiments reported in this thesis was to obtain a better understanding of the effect of ammonia treatment in combination with or without supplementation on intake and digestion of straw. Wheat straw acted in the experiments as a model for straw in general. Study of the changes in physiological parameters concerning rumen degradation and passage, comminution through rumination and during ingestion, and small intestinal protein availability as a result of ammonia treatment and/or supplementation, could help to identify processes limiting digestible energy intake from low-quality feeds. Knowlegde of factors limiting utilization of straws could contribute to the formulation of rations in which the straw is utilized optimally.

Chapter two describes the effects of supplementation of urea to low-quality feeds to increase the nitrogen availability for rumen microbes. Chapter 2.1 deals with the effects of nitrogen availability with or without additional mineral and/or energy supplementation for rumen microbes on *in vitro* organic matter degradation. Chapter 2.2 describes the effects of nitrogen availability for rumen microbes on intake and digestion of wheat straw in goats.

Chapter three deals with the effects of ammonia treatment of wheat straw on intake, site and extent of cell wall digestion and kinetics of degradation and passage (Chapter 3.1) and on efficiency of rumen microbial protein synthesis and protein availability from the small intestine (Chapter 3.2) in sheep. The last part of Chapter three (Chapter 3.3) gives the results of an experiment in which intake, digestion and energy utilization from untreated and ammonia treated wheat straw were compared between sheep and cattle.

In Chapter four, the effects of additional protein supplementation to ammoniated wheat straw are given. Chapter 4.1 deals with the effects of additional supplementation of casein of a high rumen degradability and potato protein of a relatively low rumen degradability on intake, extent and site of digestion and kinetics of degradation and passage in sheep and Chapter 4.2 describes the results of the same experiment with regard to extent and efficiency of microbial protein synthesis and small intestinal availability of protein. Chapter 4.3 gives the results of an experiment with cattle fed ammoniated wheat straw with or without an additional potato protein supplement. The effects of ammonia treatment with or without the additional protein supplementation on kinetics of comminution, rumen degradation and passage are described. Based on the results of this experiment a rumen model was designed with the principal aim to validate the rate constants of degradation, passage and comminution derived from conventional methods by model estimates (Chapter 4.4).

Ultimately, in the General Discussion (Chapter five) the major findings of the

experiments described in Chapters two, three and four are discussed in an integrated manner.

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CHAPTER 2.

SUPPLEMENTATION OF UREA TO UNTREATED STRAWS

2.1 Effect of supplementary urea, glucose and minerals on the in vitro degradation of low quality feeds

2.2 The effect of rumen ammonia nitrogen concentration on intake and digestion of wheat straw by goats

2.1 Effect of supplementary urea, glucose and minerals on the in vitro degradation of low quality feeds

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Summary

Increasing levels of ammonia-N in the rumen fluid used for in vitro incubation were achieved by supplementation of the ration of the donor cows with urea and by addition of urea either with or without glucose to the rumen fluid after collection. The ration of the donor animals consisted of wheat straw (80 %) and maize silage (20%). During the second half of the experiment the basal ration was supplemented with a mineral mixture. Wheat straw, Guinea grass and two rice straw varieties were incubated with the various kinds of rumen fluid. Parameters studied were: solubility, apparent organic matter disappearance after 48 hours of incubation (OMD_{48}), rate of organic matter degradation from 0 to 24 hours of incubation (k_1) and from 24 to 96 hours (k_2). The concentration of ammonia-N in the rumen fluid at which 95 % of the maximal OMD_{48} and k_1 were reached (88.2 and 100.0 mg/l) were independent of the feed. With regard to the k_2 the required ammonia-N concentration to reach 95 % of the maximal k_2 differed between feeds. Mineral supplementation increased OMD_{48} and k_1, but not the solubility and k_2. Glucose addition in combination with urea had no beneficial effect compared to urea supplementation alone.

Keywords: In vitro degradation, low quality feeds, urea supplementation, mineral supplementation, glucose supplementation.

Introduction

Rumen microbes utilize ammonia-nitrogen (AN) for synthesis of microbial protein, but also for processes involved in degradation of feed. The optimal AN concentration for maximal microbial growth is in the range of 20-50 mg/l (Satter and Slyter, 1974). Others reported much lower requirements for optimal growth rates of pure cultures of rumen microbes (Hespell and Bryant, 1979; Schaeffer et al., 1980) varying from 1.4 to 14 mg AN per liter. For optimal degradation of feed higher AN concentrations are required (Mehrez et al., 1977; Erdman et al., 1986). Erdman et al. (1986) reported AN requirements for optimal degradation ranging from 40 to 250 mg/l dependent on the potential digestibility of the feed.

Supplementation of urea to rations low in N may increase the intake and the digestibility of dry matter, but in some cases the effect is small and insignificant (Campling et al., 1962; Ørskov and Grubb, 1978; Kellaway and Leibholz, 1983; Dias-da-Silva and Sundstøl, 1986; Schiere and Wieringa, 1988). The effect of urea supplementation on intake and digestibility could be dependent on the potential digestibility of the feed as suggested by Ørskov and Grubb (1978).

The objectives of the present experiments were:

(1) to study the effect of AN level of the substrate on in vitro degradation of low quality feeds.

(2) to study the effect of additional minerals and easily available carbohydrates on in vitro degradation of low quality feeds.

Material and Methods

Animals, basal diet and experimental design.

Two Dutch Friesian, non-lactating cows cannulated with a large, 10 cm inside diameter, rumen cannula (Bar Diamond Inc. Parma, ID, USA) were used. The cows were individually tethered in tie stalls at the Institute for Animal Feeding and Nutrition Research at Lelystad The Netherlands and had *ad libitum* access to water. The basal diet consisted of chopped (2 cm) wheat straw (80 % on dry matter basis) and maize silage (20 % on dry matter basis). The composition of these feeds is given in Table 1. At 5.30 a.m. 40% of the ration was offered, the remaining part at 17.00 p.m. Previous to the experiment the animals were fed the basal ration for approximately 4 months. It was assumed that by that time full adaptation to the ration had been achieved.

Table 1. Chemical composition and in-vitro estimate of in-vivo digestibility of the basal ration.

Feed	Wheat straw	Maize silage	Total ration[1]
DM (%)	95.3	23.7	59.5
OM (% in DM)	86.5	93.2	87.8
CP (% in DM)	2.4	9.6	3.8
OMD (%)[2]	40.5	71.0	46.6

1 80 % wheat straw, 20% maize silage
2 Organic Matter Digestibility. Measured in-vitro (two stage Tilley and Terry) with calibration line for relation in-vitro vs. in-vivo for comparable feeds.

The experiment consisted of 4 periods. During the first period, one of the cows received the basal ration. The other received the same ration supplemented with urea (2% of DM). In period 2 the rations of the cows were exchanged. During period 3 one of the cows received the basal ration supplemented with 236 grams of a commercial mineral mixture containing Ca, P, Mg, Na, Cl and S, micro-elements and vitamines A and D, while the other animal received the basal ration supplemented with urea (2 % of DM) and 236 grams of the mineral mixture and 35 grams of sodium-sulphate to get a N/S-ratio of 10/1. In period 4 the diets of the animals were exchanged. Each period had a duration of 42 days of which 24 days were adaptation period. Rumen fluid (2 liters of each animal) was collected at day 25 and 32 of each period at 7 a.m., 1.5 hours after feeding. The rumen fluid was collected in preheated thermosflasks (39 °C) and transported to Wageningen, where it arrived about 1 hour after collection. The average temperature of the rumen fluid at arrival in Wageningen was 34 °C.

Four test feeds, wheat straw, guinea grass and two rice straw varieties were incubated with the rumen fluid. The composition of these feeds is given in Table 2. All feeds except the wheat straw were collected in Sri Lanka. The wheat straw was the same as fed in the basal ration of the donor animals. All test feeds had a high cell wall and a low N content.

The feeds were ground to pass a 1 mm sieve and 500 mg of air dry matter was weighed into 150 ml bottles. The rumen fluid of each cow was strained through four layers of cheese cloth after which it was added to a carbon dioxide saturated sodium bicarbonate buffer (Tilley and Terry, 1963) at a temperature of 39 °C. The concentration of rumen fluid in the buffer solution was 20 % and 50 ml of the rumen fluid/buffer solution was added to the feed samples in the bottles. After addition of the rumen fluid/buffer solution, the bottles were closed with a rubber stopper with a valve to let fermentation gasses out and to prevent influx of oxygen. The bottles were put in an incubator at 39 °C and shaken twice daily.

Table 2. Chemical composition and in-vitro estimate of in-vivo digestibility of the test feeds.

Feed	Wheat straw	Guinea grass[1]	Rice straw BG297-2[1]	Rice straw BG298-2[1]
DM (%)	95.3	94.7	95.0	94.1
OM (% in DM)	86.5	90.5	85.6	83.6
CP (% in DM)	2.4	4.4	4.2	3.6
NDF (% in DM)	78.3	76.3	71.4	71.0
ADF (% in DM)	49.4	52.0	43.5	43.0
Lignin (% in DM)	5.5	7.6	3.4	3.5
OMD (%)[2]	40.5	42.5	55.7	48.7

[1] Feeds from Sri Lanka. The Guinea grass was harvested at a mature stage (about 10 weeks regrowth).

[2] Measured in-vitro (two stage Tilley and Terry) with calibration line for relation in-vitro vs. in-vivo for comparable feeds.

To study the kinetics of degradation, incubation was terminated after 0.5, 24, 48 and 96 hours by filtering over glass filter crucibles (Duran no 2; poresize 40-100 μm). The residues were washed with warm water. The incubation period of 0.5 hours was applied to study the solubility of the test feeds.

The organic matter residue after incubation of t hours (OMR_t) was calculated from the weight of the crucible plus contents after drying minus the weight of the crucible plus contents after ashing and was corrected for a blank. The OMR_t was expressed as percentage of the organic matter quantity in the sample before incubation. The apparent organic matter disappearance at time t (OMD_t) was equal to (100 - OMR_t).

The following supplements were added to the test feeds in the bottles just before addition of the rumen fluid/buffer solution:

- none
- 7.5 mg urea (Merck 8486)
- 7.5 mg urea and 35.3 mg glucose (Merck 8342).

Glucose was added to study whether easily available energy would affect the effect of urea supplementation.

Within each of the four periods there were two runs of in vitro incubation. In the first run no supplement and the urea supplementation were examined and in the second run no supplement and the combined urea plus glucose supplement.

The AN content of the substrate, when no urea supplement was added to the bottles was equal to the concentration of AN in the rumen fluid divided by 5, since there was 20% of rumen fluid in the substrate. The amount of urea added to the substrate for the in-vitro incubation increased the N-content of the substrate with 70 mg/l. All substrates in which test feeds were incubated are given in Table 3. Within substrates a large variation in AN content was found. AN content was therefore treated as a continuous variable for statistical analysis.

Table 3. AN level in the various substrates, in which the test feeds were incubated (means and standard deviation (s.d.)).

Treatments				AN level (mg/l)	s.d.
Cow diet		In-vitro supplement			
Urea	*Minerals*	*Urea*	*Glucose*		
-	-	-	-	12.8	4.72
+	-	-	-	47.9[1]	7.49
-	+	-	-	9.3	2.53
+	+	-	-	41.9[1]	14.20
-	-	+	+ and -	82.8[2]	4.72
+	-	+	+ and -	117.9[2]	7.49
-	+	+	+ and -	79.3[2]	2.53
+	+	+	+ and -	111.9[2]	14.20

[1] AN level due to in-vivo supplementation of urea.
[2] AN level due to in-vivo and in-vitro urea supplementation. Value is equal to the level due to in-vivo supplementation plus 70 mg/l due to in-vitro supplementation of urea.

Dry matter, ash and Kjeldahl-N were determined by standard methods. Cell wall analysis was done by the methods of Goering and Van Soest (1970). Samples for analysis of the composition of the rumen fluid were taken immediately after arrival in the laboratory. Measured were pH, total N content by standard Kjeldahl procedure, AN by distillation and titration (i.e. the last part of the Kjeldahl procedure) and volatile fatty acids by gas chromatography.

The rate of degradation of the organic matter, defined as the percentage of total organic matter apparently degraded per hour was calculated for the time intervals from 0 to 24 hours (k_1) and from 24 to 96 hours (k_2) by the formulas:

$k_1 = (OMD_{24} - OMD_{0.5})/24$

$k_2 = (OMD_{96} - OMD_{24})/72$

Analysis of variance and covariance was done with SAS computer programmes (SAS, 1985). Full models used for statistical analysis were:

(1) $Y_{ijk} = mean + M_i + U_j + M_i * U_j + e_{ijk}$ for analysis of the effect of in vivo mineral supplementation (M) and urea supplementation (U) on the various parameters concerning the composition of the rumen fluid (Y). The number of data in each analysis was 16.

(2) $Y_{ijklmn} = mean + M_i + G_j + F_k + C_l + b * AN_m + c * AN_m * AN_m$ + two-way interactions + e_{ijklmn} for analysis of the effect of glucose as an additional supplement to urea supplementation in vitro. M is the mineral effect, G the glucose effect, F the feed effect, C the cow effect and AN the AN level (mg/l) of the substrate and Y is either k_1, k_2 or OMD_{48}. Two-way interactions were only included if significant. Only the results from incubations with in vitro supplementation of urea and urea plus glucose were

analysed. The number of data was 56. Eight values were missing.
(3) Y_{ijklm} = mean + M_i + F_j + C_k + b * AN_l + c * AN_t * AN_l + two-way interactions + e_{ijklm} for analysis of the effect of mineral supplementation (M), the feed effect (F), the cow effect (C) and the AN level of the substrate (AN mg/l) on the degradation parameters (Y). Two-way interactions were only included if significant. The number of data for this analysis was 112. Sixteen values were missing.

Significant differences existed neither between period 1 and period 2 nor between period 3 and period 4 with regard to in vitro degradation parameters. Therefore, it was assumed, that there was also no difference between the first two and the last two periods. The period effect was therefore not included in the statistical models.

Results

Composition of the rumen fluid.

The composition of the rumen fluid as affected by the rations of the donor animals is given in Table 4. The pH of the rumen fluid was significantly reduced by mineral addition, but was not affected by urea supplementation. The volatile fatty acid (VFA)-content of the rumen fluid increased due to urea and mineral supplementation. The C2/C3-ratio was lowest, when no supplement was added. There was no difference between the C2/C3-ratios, when the donor animals received urea, minerals or both supplements. AN and non ammonia-N content (NAN, total N minus AN) were significantly increased when urea was added to the ration of the donor animals. Data concerning intake and rumen parameters of the donor animals will be published elsewhere.

Table 4. Composition of the rumen fluid collected 1.5 hour after feeding as affected by supplementation of the donor animals.

Ration	Without urea		With urea		Significances		
	-M[1]	+M[2]	-M	+M	Urea	M	Urea*M
pH	7.10	6.90	7.22	6.88	ns	*	ns
VFA (mmol/l)	68.9	76.8	73.8	80.5	*	*	ns
C2/C3-ratio	2.92	3.74	3.51	3.54	ns	*	*
NH_3-N (mg/l)	64.0	43.0	239.3	210.8	***	ns	ns
NAN (mg/l)	151.8	169.4	192.4	233.8	*	ns	ns

* p < 0.05, *** p < 0.001
[1] no mineral supplementation
[2] mineral supplementation

STELLINGEN

1. De vrijwillige opname van verteerbare organische stof uit laagwaardige ruwvoeders door herkauwers wordt *niet* primair beperkt door de verwerkingscapaciteit van het pens-netmaag-complex.

   Dit proefschrift.

   Ketelaars, J.J.M.H. and B.J. Tolkamp, 1992. Toward a new theory of feed intake regulation in ruminants 1. Causes of differences in voluntary feed intake; critique of current views. Livestock Production Science 30: 269-296.

2. De fractionele verteringssnelheid van potentiëel verteerbaar voer in de pens wordt onderschat met de *in sacco* methodiek en de fractionele verdwijningssnelheid van kleine deeltjes uit de pens wordt overschat wanneer deze gebaseerd is op het uitscheidingspatroon van merkstoffen in de faeces.

   Dit proefschrift.

   Aitchisson, E., M. Gill, J. France and M.S. Dhanoa, 1986. Comparison of methods to describe the kinetics of digestion and passage of fibre in sheep. Journal of the Science of Food and Agriculture 37: 1065-1072.

3. De bijdrage van microbiële fermentatie in het blinde darm/dikke darm-complex van herkauwers aan de totale celwandvertering is gering.

   Dit proefschrift.

4. Bij *ad libitum* gevoerde rantsoenen lijkt de kwaliteit van het rantsoen geen effect te hebben op de efficiëntie van benutting van zowel metaboliseerbare energie als van werkelijk uit de dunne darm geresorbeerd eiwit.

   Dit proefschrift.

   Tolkamp, B.J. and J.J.M.H. Ketelaars, 1993. The effect of ad libitum feeding on the efficiency of energy utilization in growing and lactating cattle. Animal Production 56: 431-432.

5. Onder omstandigheden waarin toevoeging van een energiesupplement of onbestendig eiwit aan een rantsoen gebaseerd op laagwaardige ruwvoeders resulteert in volledige substitutie van de verteerbare organische stof opname van het ruwvoeder door die van het supplement kan toevoeging van bestendig eiwit de totale opname van verteerbare organische stof verhogen.

   Dit proefschrift.

6. Pensmodellen verschaffen geen inzicht in causale verbanden tussen voeropname en verdwijning van materie uit de pens.

7. De grootste uitdaging voor fundamenteel onderzoek op het gebied van de voeding van één- en meermagigen is de regulering van de voedselopname.

8. Bij de veredeling van graangewassen voor teelt in tropische gebieden zouden zowel de kwaliteit als de kwantiteit van het stro onderdeel van de selectiecriteria moeten zijn.

9. Het feit, dat werknemers in de gezondheidszorg een hoog ziekteverzuim hebben is tekenend voor de arbeidsomstandigheden in deze sector.

10. De marginalisatie van tropische richtingen aan de Landbouwuniversiteit door fusies en stille liquidaties is in strijd met haar op internationalisering gerichte beleid.

11. De duurzaamheid van ontwikkelingsprojecten is dikwijls groter dan die van de beleidsdoelstellingen van het Ministerie van Ontwikkelingssamenwerking die aan deze projecten ten grondslag liggen.

12. Overtreding van het aanlijngebod voor honden is eerder regel dan uitzondering.

13. Vergelijking van de speelsterkte van damcomputers met die van menselijke dammers is even zinloos als elke andere vergelijking tussen mensen en machines.

Stellingen behorend bij het proefschrift: Wheat straw as ruminant feed: effect of supplementation and ammonia treatment on voluntary intake and nutrient availability, Simon J. Oosting, 10 november 1993.

Effect of additional glucose on degradation parameters.

The effect of glucose addition was not significant as is illustrated in Table 5 in which the least square means (lsmeans) and the standard error of lsmeans (SEM) of the degradation parameters for urea plus glucose addition and for urea supplementation alone are given. For further analysis of the data no distinction was made between these two treatments.

The effect of AN level and mineral level of the substrate on in vitro degradation parameters.

OMD_{48} and k_1 were higher when the rumen fluid in which the test feeds were incubated was derived from donor animals that received a mineral supplement (see Table 6). The k_2 and $OMD_{0.5}$ were not affected by this mineral supplementation. The interaction between mineral supplementation and AN concentration in the substrate was not significant, indicating that the positive effect of mineral supplementation on OMD_{48} and k_1 was independent of AN level.

Table 5. Least square means of degradation parameters for in-vitro incubation with addition of urea and urea plus glucose to the substrate (between brackets SEM).

	Urea	Urea plus glucose
OMD_{48} (%)	40.2 (0.69)	39.1 (0.70)
k_1 (%/h)	0.81 (0.018)	0.77 (0.021)
k_2 (%/h)	0.33 (0.011)	0.32 (0.013)

Table 6. Least square means of degradation parameters of test feeds incubated with rumen fluid from animals fed the basal ration with or without supplementary minerals. (Between brackets SEM).

	No minerals	Minerals
$OMD_{0.5}$ (%)	7.0 (0.26)	7.5 (0.28)
k_1 (%/h)	0.55[a] (0.015)	0.67[b] (0.016)
k_2 (%/h)	0.28 (0.009)	0.28 (0.011)
OMD_{48} (%)	30.2[a] (0.47)	34.8[b] (0.49)

Different superscripts per row indicate significant differences ($p < 0.05$).

A significant feed effect was observed for all degradation parameters. Guinea grass had the highest solubility ($OMD_{0.5}$). The rice straw varieties were intermediate and did not differ. The wheat straw had the lowest solubility (see Table 7). The difference between feeds with regard to k_1 were small. Guinea grass differed significantly from the rice straw varieties. All feeds differed from each other with regard to k_2. Rice straw BG297-2 had the highest rate of degradation in the period from 24 to 96 hours after start of incubation and guinea grass was least degraded during this period. The k_2 was lower than the k_1 and was 43.7 %, 32.9 %, 55.1 % and 51.2 % of the k_1 for wheat straw, guinea grass, rice straw BG297-2 and rice straw BG298-2, respectively. OMD_{48} was significantly lower for wheat straw than for the other feeds.

The relation between OMD_{48} or k_1 and the AN level of the substrate is given in Table 8. The interaction between feed and AN level was not significant for these two degradation parameters, which indicates that for all feeds the maximal value of the degradation parameter was found at the same AN level. The required AN level for the maximum value of degradation parameters is also given in Table 8 as well as the required AN level to reach 95 % of maximal degradation, which was 88.2 mg AN/l for OMD_{48} and 100.0 mg AN/l for k_1.

Table 7. Least square means of degradation parameters of the four test feeds (between brackets SEM).

	Wheat straw	Guinea grass[1]	Rice straw BG297-2[1]	Rice straw BG298-2[1]
$OMD_{0.5}$ (%)	3.3^{a} (0.42)	11.3^{c} (0.41)	7.4^{b} (0.36)	7.0^{b} (0.35)
k_1 (%/h)	0.61^{ab} (0.021)	0.56^{a} (0.021)	0.66^{b} (0.020)	0.63^{b} (0.020)
k_2 (%/h)	0.27^{b} (0.015)	0.19^{a} (0.015)	0.36^{d} (0.014)	0.32^{c} (0.014)
OMD_{48} (%)	27.4^{a} (0.67)	33.6^{b} (0.67)	34.7^{b} (0.61)	33.9^{b} (0.61)

Different superscripts per row indicate significant differences ($p < 0.05$).

Table 8. Relation between degradation parameters and AN level of the substrate (Between brackets SE of estimate).

	OMD_{48} (%)	k_1 (%/h)
Intercept	13.2 (1.21)	0.19 (0.020)
Linear regression coefficient ($*10^{-2}$)	43.5 (3.52)	0.97 (0.111)
Quadratic regression coefficient ($*10^{-4}$)	-17.5 (2.59)	-0.36 (0.085)
Required AN level for maximal degradation (mg/l)	124.3	136.1
Required AN level for 95 % of maximal degradation (mg/l)	88.2	100.0

The relation between k_2 and the AN level of the substrate was dependent on the feed. The regression equations describing this relation for each feed are given in Table 9 as well as the required AN level for maximal k_2 and for 95 % of maximal k_2. The required AN levels for maximum k_2 were lower than the required AN levels for the maximal level of the other degradation parameters. To reach 95 % of maximal k_2 guinea grass had a lower AN requirement than the other feeds.

The number of test feeds was too small to test for correlations between feed

Table 9. Relation between k_2 and the AN level of the substrate for the four test feeds (between brackets SE of estimate).

Feed	Wheat straw	Guinea grass	Rice straw BG297-2	Rice straw BG298-2
Intercept	0.14 (0.019)	0.14 (0.019)	0.14 (0.019)	0.14 (0.019)
Linear regression coefficient ($*10^{-2}$)	0.41^{b} (0.099)	0.16^{a} (0.097)	0.65^{c} (0.100)	0.58^{c} (0.101)
Quadratic regression coefficient ($*10^{-4}$)	-0.24^{ab} (0.083)	-0.10^{a} (0.081)	-0.37^{b} (0.089)	-0.35^{b} (0.091)
Required AN level for maximal degradation (mg/l)	87.4	78.5	88.2	84.0
Required AN level for 95 % of maximal degradation (mg/l)	61.2	45.3	64.2	60.0

Different superscripts per row indicate significant differences ($p < 0.05$).

composition and degradation parameters or required AN levels.

Figure 1 gives the relationships between substrate AN concentration and OMD_{48} for the individual test feeds with or without mineral supplementation to the basal ration of the donor animals of the rumen fluid. The regression equations were:

Wheat straw:
No minerals: $7.3 + 0.40*AN - 0.0014*AN^2$ ($R^2 = 0.902$, RSD = 3.21)
Plus minerals: $14.0 + 0.37*AN - 0.0013*AN^2$ ($R^2 = 0.927$, RSD = 2.90)

Guinea grass:
No minerals: $18.8 + 0.38*AN - 0.0019*AN^2$ ($R^2 = 0.874$, RSD = 2.36)
Plus minerals: $22.0 + 0.35*AN - 0.0017*AN^2$ ($R^2 = 0.974$, RSD = 1.19)

Rice straw BG297-2:
No minerals: $12.0 + 0.51*AN - 0.0021*AN^2$ ($R^2 = 0.898$, RSD = 3.69)
Plus minerals: $16.9 + 0.46*AN - 0.0017*AN^2$ ($R^2 = 0.844$, RSD = 4.71)

Rice straw BG298-2:
No minerals: $12.4 + 0.38*AN - 0.0010*AN^2$ ($R^2 = 0.850$, RSD = 4.60)
Plus minerals: $16.0 + 0.55*AN - 0.0025*AN^2$ ($R^2 = 0.942$, RSD = 2.74)

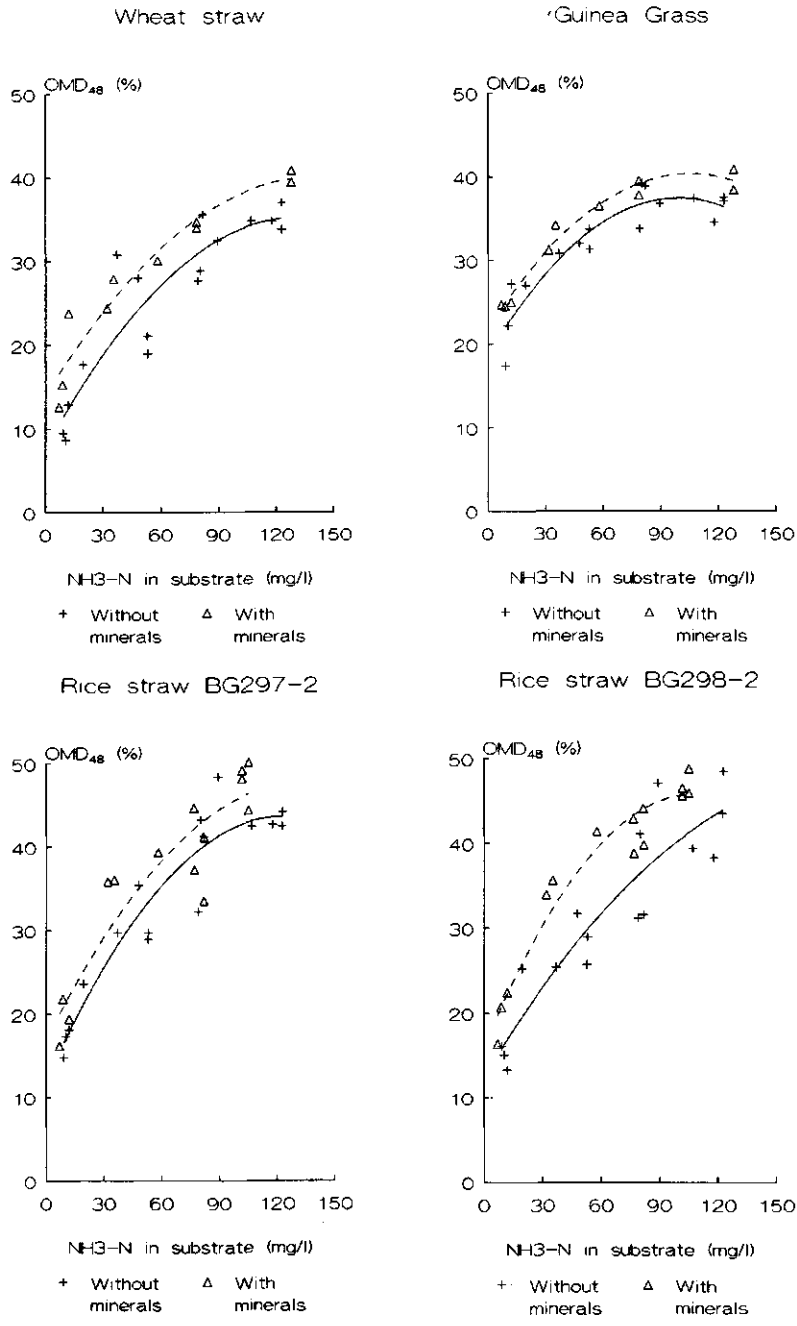

Figure 2. OMD_{48} in relation to ammonia nitrogen concentration in the substrate. (Whole line: without minerals, dotted line: with minerals)

Discussion

Urea supplementation of the ration of the donor animals increased VFA and NAN concentration of the rumen fluid, which are indications of a higher rate of digestion and a higher microbial growth. However, the higher NAN concentration measured in the rumen fluid may also be due to a higher concentration of feed nitrogen, since intake of the basal ration, when supplemented with urea was also higher (data will be published elsewhere). No relation existed neither between pH and concentration of VFA's in rumen fluid nor between pH and AN concentration. Why mineral supplementation decreased pH is unknown.

All kinds of supplementation increased the C2/C3 ratio of the rumen fluid. A possible reason could be that production rates of individual VFA's are affected by a lack of nutrients. There are indications that the content of lactic acid in the rumen of animals on a sulphur deficient diet is high due to a diminution of the acrylate pathway for conversion of lactate to propionate (Slyter et al., 1986). Other pathways may also be affected by nutrient deficiency. The phenomenon, that the C2/C3 ratio increases, when supplementory nutrients are added to a low quality ration may also explain the fact reported from Sri Lanka, that the milk fat content increased after supplementation or treatment of rice straw with urea (Ibrahim, personal communication).

Urea supplementation increased the AN content of the rumen fluid. The levels found in this experiment are in line with those observed by Erdman et al. (1986), who infused urea into the rumen of cows fed on corn meal and cotton seed hulls. The observed AN levels were, however, much lower than those observed by Mehrez et al. (1977) when they supplemented urea to a whole barley grain ration of sheep. In vitro degradation in the present experiment was positively related to the AN content of the substrate as was also reported by Mehrez et al. (1977) and Erdman et al. (1986).

No relation between potential digestibility and the required AN level for maximal k_1 or OMD_{48} could be found in the present experiment in contrast with findings of Erdman et al. (1986). This may be due to the fact that the differences between feeds in k_1 and OMD_{48} were small. The k_2, which is more determining the potential digestibility than k_1 and OMD_{48} for the feeds studied in the present experiment differed between feeds. The k_2 represents the rate of degradation of the worst digestible part of the feed. There was an indication that the required AN level to reach 95 % of the maximal k_2 was dependent on the k_2 value of the feed. The number of feeds tested was, however, too limited to analyse this relation statistically. The required AN level for 95 % of optimal k_2 seemed lower than the AN level required for 95 % of optimal OMD_{48} or k_1.

There was no effect of supplementary minerals to the donor animals on the k_2. A reason for this may be that, due to lysis of microbes and the decreasing amount of substrate during the later phase of in vitro fermentation the AN and mineral levels in the substrate are less limiting than during the first phase of in vitro fermentation.

The required AN levels for maximal degradation were high when compared to data reported by Erdman et al. (1986), who calculated a relation between potential digestibility

and required AN level for a number of feeds varying in maximal digestibility from 45 to 80 %. The relation they calculated was:
Required AN = - 157.1 + 4.51 * (potential digestibility) (R^2 = 0.50). Assuming a potential digestibility of 50 % for the feeds tested in the present experiment the required AN level should be approximately 70 mg/l, lower than the observed required AN level of 88 - 100 mg/l. The residual variation of the above given regression was large, however, making a judgment of this difference difficult.

Mineral supplementation of the donor animals did not increase the NAN level nor the AN level of the rumen fluid. The increased VFA content may, however, be an indication of a higher microbial activity. This higher microbial activity was also found when the rumen fluid of the animals that were supplemented with minerals was used for in vitro incubation. The effect of the mineral supplementation on in vitro degradation parameters may be contributed to sulphur, since all other macro elements in the mineral mixture were added in the buffer solution. There could, however, also be an effect of micro elements and/or vitamines, that were added in the mineral mixture. Effects of sulphur addition to supplementary urea are variable (McLennan et al., 1981) and may be dependent on the ratio between rumen available N and rumen available sulphur. Sulphur supplementation increased the amount of cellulolytic bacteria and the VFA production in continuous cultures as well as in the rumen of sheep and calves (Slyter et al., 1986).

Although we added a considerable amount of glucose to the substrate (15-20 % of digestible organic matter) an effect of this addition to a urea supplement could not be found. This is in contrast to what Hoover (1986) mentioned in a review about fiber digestion. In vitro as well as in vivo the fiber digestion was depressed by addition of readily fermentable carbohydrates due to preference of microbes for these carbohydrates and a reduction in pH. In the in vitro system we used the pH was buffered, however.

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2.2 The effect of rumen ammonia nitrogen concentration on intake and digestion of wheat straw by goats

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Abstract

In a 4 x 4 Latin square design the ammonia nitrogen (N) concentration in the rumen of West African Dwarf goats was manipulated by infusion of urea at levels of 0, 1.68, 3.36 and 5.04 g N/day. Sulphur (S) was included in the infusate at an N/S ratio of 10/1. The resulting ammonia N concentrations in the rumen were 39.8, 96.5, 180.1 and 250.2 mg/l for the respective levels of N infusion. Intake increased significantly with infusion of N, but did not differ significantly between infusion levels of 1.68, 3.36 and 5.04 g/day. N infusion did not affect digestion of any nutrient except N, the apparent digestion of which increased with increasing level of N infusion. The concentration of volatile fatty acids in the rumen increased with increasing level of digestible organic matter intake ($r = 0.63$), without a significant change in rumen pH. No effect of N infusion on molar composition of the rumen volatile fatty acid pool was observed. The osmolality of the rumen fluid was significantly correlated with rumen ammonia N concentration ($r = 0.64$). Rate of passage of the particulate phase increased significantly with increasing N infusion, whereas the rate of passage of the fluid phase and the rumen volume were not significantly affected by N infusion. The rate of degradation, the potential degradable fraction and the disappearance after 48 h of in sacco incubation of neutral detergent fiber were not significantly affected by treatment.

Introduction

The ammonia N concentration in the rumen fluid required to assure maximum microbial growth in vitro has a minimum value of 20-50 mg/l (Satter and Slyter, 1974). For maximal degradation of feed by rumen microbes a higher minimum required rumen ammonia N concentration was reported (Mehrez et al., 1977; Erdman et al., 1986; Oosting et al., 1989). The required ammonia N concentration for maximal degradation depends probably on the potential degradability of the feed (Ørskov and Grubb, 1978; Erdman et al., 1986). Oosting et al. (1989) observed that for maximal in vitro degradation of low quality feeds, ammonia N concentrations in the range of 60-100 mg/l were required, higher than the ammonia N concentration often observed in animals fed low quality feeds without any supplement.

Urea supplementation to low-quality feeds to increase the rumen availability of nitrogen could therefore increase the rumen degradation and energy intake. The objectives of the present experiment were: 1) to study the effect of increasing the ammonia N concentration in the rumen by urea infusion on digestion and voluntary intake of wheat straw by West African Dwarf goats; 2) to study whether the expected increased intake is to be attributed to increased rumen degradability or alternatively to increased rate of passage from the rumen and/or an increased rumen capacity.

Materials and methods

Four mature West African Dwarf wether goats with an average weight of 26.9 kg were fitted with a small PVC rumen cannula in the dorsal rumen sac. The wethers were kept in metabolism cages during the experiment and were fed twice daily, at 08.30 and 16.00 h. Water was freely available. Wheat straw, chopped to a length of approximately 5 cm, was fed ad libitum (1500 g daily) starting 3 weeks before the first experimental period. The composition of the wheat straw is given in Table 1.

The ammonia N concentration in the rumen fluid was manipulated by daily infusion into the rumen of approximately 1.5 liter water containing 0, 2.4, 4.8 or 7.2 g urea per liter. Sulphur (S), supplied as sodium sulphate was added to the infusate to give a N/S-ratio of 10:1. The solution was freshly prepared each day and was infused into the rumen by a peristaltic pump. The infusion started two weeks before the first experimental period.

The experiment was designed as a 4 x 4 Latin square. Each experimental period had a duration of 14 days. The first three days of each experimental period were for adaptation of the animals to the new infusion level. A period of three days was chosen based on the observation during the adaptation period before the experiment started that feed intake stabilized three days after the start of the infusion.

Table 1. Composition of the wheat straw (g/kg)

DM	914
Ash[1]	74
NDF[1]	815
Hemicellulose[1]	299
Cellulose[1]	431
Lignin[1]	85
Nitrogen	6.5

[1] g/kg DM

On Days 4 and 5 rumen fluid samples were taken at 4 h intervals starting at 08.00 h untill 08.00 h the next morning. Samples were analysed for pH, osmolality, volatile fatty acids (VFA) and ammonia N. The pH and osmolality were measured immediately after taking the samples. Osmolality was determined with a Knauer osmometer and VFA by gas-liquid chromatography (Packard Becker 419 (Chrompack, Bergen op Zoom, Netherlands), 6 ft. glass column of 2 mm i.d., filled with 80-100 mesh Chromosorb 101 (Chrompach, Bergren op Zoom, Netherlands), carrier gas N_2, saturated with formic acid; 190°C). The ammonia N concentration was determined by the indophenol method (Scheiner, 1976) with a Hitachi (Tokyo, Japan) u2000 spectrophotometer at a wavelength of 634.8 nm.

Intake and digestion were measured for 5 days starting on Day 4. Daily feed residue and faeces were collected and dried at 70°C for 24 h and ground. After pooling the samples for each animal, they were stored pending further analysis. Dry matter (DM) and ash were determined by standard procedures at 103°C and 550°C, respectively, and N was determined by the Kjeldahl method. Cell wall analysis was done according to Goering and Van Soest (1970). Hemicellulose was calculated as neutral detergent fiber (NDF) minus acid detergent fiber (ADF) and cellulose as ADF minus acid detergent lignin (ADL).

On Day 9, at 08.00 h, 50 ml of a Co-EDTA solution (0.5 % Co) and 5 g Cr-NDF (4.9 % Cr), both prepared according to Udén et al. (1980) were introduced into the rumen via the fistula. Rumen fluid samples for Co analysis were taken 2, 4, 6, 8, 10, 14, 24, 28 and 36 h after introduction of the markers into the rumen. Faecal samples were collected 8.5, 14, 24.5, 30.5, 36.5, 48.5, 54.5, 60.5, 72.5, 78.5, 84.5, 96.5, 102.5, 108.5, 120.5, 126.5 and 132.5 h after adding the Cr-NDF to the rumen. Co and Cr were determined after wet destruction by atomic absorption spectrophotometry (Varian (Palo Alto, CA, USA) SpectraA 300) at wavelengths of 240.7 and 357.9 nm, respectively. The fractional rate of passage of the fluid phase and the rumen fluid volume were estimated from the following model:

$$\ln([Co_t]) = \ln([Co_0]) - k_l * t,$$

where $[Co_t]$ is the Co concentration in rumen fluid at time t and k_l is the fractional rate of passage of rumen fluid. From the estimate of $[Co_0]$ the rumen fluid volume was estimated

as:

rumen fluid volume = $Q/[Co_0]$,

where Q is the quantity of Co introduced into the rumen. The fractional rate of passage of the particulate phase (k_p) was derived from the logarithmic decline in Cr concentration in the descending part of the excretion curve (Grovum and Williams, 1973).

In sacco incubation was done with dacron bags (pore size 40 μm) containing 2 g of ground (1 mm sieve) wheat straw. One bag was incubated on Day 9 in each rumen and removed after 48 h on Day 11. The replicate was incubated on Day 11 and removed on Day 13. The fraction disappearing at t = 0 was estimated by incubating bags for 5 minutes on Day 13 in duplicate. At the end of the last experimental period the animals remained in the metabolism cages and were kept at the same rate of infusion as during the final period, and two dacron bags containing 2 g of ground wheat straw were incubated in each rumen for 240 h to determine the truly undegradable fraction. After collection, the bags were washed in tap water and boiled with neutral detergent (Goering and Van Soest, 1970) for 1 h to determine the NDF residue.

From the estimates of degradation after 0, 48 and 240 h the potentially degradable (D) and truly undegradable (U) fraction and k_d (fractional rate of degradation) were calculated by the following formulae based on the general first order degradation model as given by Robinson et al. (1986):

$R_t = U + D * e^{-kd*t}$, where R_t is the residue at time t.
$U = R_{240}$.
$D = R_0 - R_{240}$.
$k_d = - \ln((R_{48} - U)/D)/48$.

The data were statistically analysed with the program DBSTAT (Brouwer, 1990) and the model:

$$Y_{ijklm} = \mu + Period_i + Animal_j + Treatment_k + PT_l + error_{ijklm},$$

where PT is the treatment in the preceding period. This factor was put into the model to correct for a carry-over effect related to the infusion level in the preceding period. In the experimental design each treatment was followed by every other treatment. The treatment in the period preceding the first experimental period was similar to that in the first experimental period.

Results

Carry-over effects caused by the treatment in the preceding period were not significant, except for the rate of passage of the particulate phase. In Table 2, the average daily ammonia N concentration and the daily intake (I) of DM, organic matter (OM), NDF and N is given. The ammonia N concentration increased with increasing urea N infusion level. DMI, OMI and NDFI increased as a result of supplementation, but no significant increase in intake could be observed when the rumen ammonia N concentration increased beyond 96.5 mg/l. Although DMI as proportion of offered DM differed between treatments (varying from 28 to 35 %), the level of excess offered was sufficiently high to allow maximum selection for all treatments.

Table 3 shows the digestion of various feed components as affected by urea infusion or rumen ammonia N concentration. No significant differences were found between treatments, except for digestion of N. The Lucas equation (Van Soest, 1982) was used to relate N digestibility to DMI:

$$DNI = -a + b*NI,$$

where DNI and NI are digestible N intake as percentage of DMI and N intake as percentage of DMI, respectively, a is the metabolic faecal N loss (MFN, g/100 g DMI) and b is the true digestibility of N. Estimates for the MFN and true N digestibility were 0.596 g/100 g DMI and 0.998, respectively (R^2 = 0.984, n = 16). Cellulose was significantly more digestible than hemicellulose. Digestible organic matter intake (DOMI) increased as a result of N supplementation, but did not increase beyond a supplementation level of 1.68 g N/day.

Table 2. Rumen ammonia N concentration and intake of DM, OM, NDF and N.

N-infusion level (g/day)	0	1.68	3.36	5.04	SEM
Rumen ammonia N concentration (mg/l)	39.8^a	96.5^b	180.1^c	250.2^d	8.29
Intake ($g/kg^{0.75}/d$)					
DM	32.3^a	39.3^b	40.9^b	40.0^b	1.38
OM	29.7^a	36.2^b	37.6^b	37.3^b	1.26
NDF	25.5^a	31.1^b	32.9^b	32.2^b	1.18
N					
- *straw*	0.23^a	0.27^b	0.28^b	0.28^b	0.007
- total	0.23^a	0.42^b	0.56^c	0.70^d	0.015

Different superscripts in a row indicate significant differences ($P < 0.05$).

Table 3. Digestion of wheat straw components (g/kg) and digestible organic matter intake (DOMI, g/kg$^{0.75}$/day).

N-infusion level (g/day)	0	1.68	3.36	5.04	SEM
DM	435	440	430	432	8.8
OM	430	443	430	438	9.4
NDF	488	500	500	499	12.1
Hemicellulose	475	473	495	506	19.7
Cellulose	558	583	578	563	15.6
Lignin	120	200	115	166	8.7
Nitrogen	158^{a}	435^{b}	548^{c}	643^{d}	26.4
DOMI	12.7^{a}	16.1^{b}	16.2^{b}	16.4^{b}	0.75

Different superscripts in a row indicate significant differences ($P < 0.05$).

The rate of passage of the particulate phase (k_p) tended ($P < 0.10$) to vary between 0 and 1.68 g of N supplementation and differed significantly between the two lowest and the two highest levels of supplementation (Table 4). A significant carry-over effect of the treatment in the preceding period on k_p was observed: with a higher infusion level in the preceding experimental period the k_p in the following period was higher. The rate of passage of the fluid phase and the rumen volume did not differ between treatments. Table 4 also gives the in sacco degradation parameters. No significant differences in D, U, degradation after 48 h of incubation and k_d were observed between treatments.

Table 4. Rate of passage of particulate (k_p) and fluid (k_l) phase, rumen volume, and rate of degradation (k_d), potentially degradable fraction (D) and truly undegradable fraction (U) of NDF and NDF disappearance after 48 h of incubation in nylon bags ($NDFD_{48}$).

N-infusion level (g/day)	0	1.68	3.36	5.04	SEM
k_p (%/h)	2.23^{a}	2.30^{a1}	2.56^{c}	2.56^{c}	0.017
k_l (%/h)	5.75	6.00	6.18	5.44	0.167
Rumen volume (l)	6.37	6.32	6.32	7.46	0.424
k_d (%/h)	2.0	1.7	2.4	1.8	0.28
D (%)	43.6	41.7	43.0	43.0	0.90
U (%)	39.9	40.3	40.3	40.3	0.88
$NDFD_{48}$	43.0	41.3	45.5	41.6	1.83

Different superscripts in a row indicate significant differences ($P < 0.05$).
1: $p < 0.10$

Table 5 shows the rumen fluid parameters. The osmolality of the rumen fluid increased with increasing rumen ammonia N concentration ($r = 0.64$, $p < 0.01$, $n = 16$). The VFA concentration increased as a result of N supplementation, but did not increase further when the supplementation level was higher than 1.68 g N/day. The VFA concentration in the rumen fluid was significantly correlated with DOMI ($r = 0.63$, $p < 0.01$, $n = 16$). The pH and the molar composition of the VFA in the rumen did not differ significantly between treatments. The non-glucogenic/glucogenic ratio, which is equal to the glucogenic equivalent of the non glucogenic VFA's acetate and butyrate (HAc + 2*HBu) divided by the concentration of propionate, which is glucogenic, did not differ between treatments. Only traces (less than 0.5 mmol/l) of iso-butyrate and valeriate could be found in the rumen fluid for all treatments.

Table 5. Osmolality (milli-osmoles/l), pH, concentration of volatile fatty acids (VFA, mmol/l) and molar proportions (mol/100 mol VFA) of acetate (HAc), propionate (HPr) and butyrate (HBu) and non-glucogenic/glucogenic ratio (NGR).

N-infusion level (g/day)	0	1.68	3.36	5.04	SEM
Osmolality	233^{a}	247^{ab}	255^{ab}	270^{b}	7.7
pH	6.54	6.50	6.47	6.53	0.034
VFA concentration	64.2^{a}	76.4^{b}	78.4^{b}	79.3^{b}	3.50
Molar proportions:					
HAc	79.4	79.6	79.3	79.3	0.60
HPr	17.0	16.5	16.8	16.9	0.37
HBu	3.6	3.9	3.9	3.9	0.33
NGR	5.2	5.4	5.2	5.2	0.12

Different superscripts in a row indicate significant differences ($P < 0.05$).

Discussion

Urea supplementation to low quality feeds may affect DMI and dry matter digestion (DMD) as reported by Campling et al. (1962) for oat straw and by Dias-da-Silva and Sundstøl (1986) for wheat straw. Increases in intake of other low quality feeds as paspalum hay and rice straw as a result of urea supplementation were reported by McLennan et al. (1981) and Kellaway and Leibholz (1983). Ørskov and Grubb (1978) found only small and insignificant increases in DMI and DMD of untreated barley straw supplemented with urea in sheep. However, DMI and DM digestibility increased significantly, when urea was supplemented to NaOH treated barley straw, indicating that the potential digestibility of the

basal ration is one of the determinants of the required N availability in the rumen.

In an in vitro experiment with wheat straw, guinea grass and rice straw, Oosting et al. (1989) estimated the required rumen ammonia N concentration for maximal degradation of the feeds to be in the range of 60-100 mg/l. Erdman et al. (1986) regressed minimum required ammonia N concentration in rumen fluid on maximum digestibility of feedstuffs and obtained the following equation :

Minimum rumen ammonia N concentration (mg/l) = 4.52 * maximum digestibility (%) - 157.1 (R^2 = 0.50).

For the wheat straw in the present experiment this would mean, that the minimally required ammonia N concentration in the rumen fluid would be around 70 mg/l, assuming a potential true digestibility of 50 %.

In the present experiment, however, no effect of urea supplementation and increased rumen ammonia N concentration on digestion was observed, although the rumen ammonia N concentration of unsupplemented wheat straw was only 39.8 mg/l. A possible explanation is that the ammonia N concentration in the rumen fluid, without urea supplement, was already sufficient for maximum rumen degradation. However, intake and k_p increased as a result of urea supplementation in the present experiment. A higher k_p results in a shorter retention time in the rumen and consequently a lower rumen degradation of NDF. However, no difference in whole tract NDF digestion was observed, which could mean that either the rate of NDF degradation in the rumen or the contribution of the hindgut to whole-tract NDF digestion was higher when urea was added to the wheat straw. The higher rumen concentration of VFA's observed for urea supplemented wheat straw could be indicative of a higher rate of degradation in the rumen. However, this could not be confirmed by the in sacco data, which indicated that for NDF neither k_d nor degradation after 48 h of incubation differed significantly between treatments. The conclusion seems justified, therefore, that the increased intake could not be attributed to an increased rumen degradation of NDF.

Increased intake may be associated with higher total rumen contents, a higher DM content in the rumen fluid or a higher k_p, or a combination of these factors (Owens and Goetsch, 1986). Estimation of the rumen fluid pool by Co-EDTA as a marker did not show significant differences in rumen pool size, but the DM concentration in the rumen could have increased. Doyle and Panday (1990) observed a higher rumen DM fill for urea-supplemented than for unsupplemented wheat and rice straw. The k_p increased as a result of urea supplementation in the present experiment. The observations that a carry-over effect of the treatment in the preceding period was found for k_p, but not for OMI or DOMI and that the correlation between DOMI and k_p was not significant (0.14 if not corrected for the animal effect and 0.41 if corrected) indicated that k_p and DOMI were not strongly associated.

The increased k_p should therefore probably not be regarded as the determinant of the higher intake as a result of urea supplementation, but as an associated factor. Egan and Doyle (1985), for an oaten hay based diet, and Doyle and Panday (1990), for wheat and rice straw,

attributed the increased intake of sheep related to urea and sulphate infusion to the increased availability of small intestinal digestible crude protein. The increased small intestinal protein availability was probably caused by an increased microbial protein synthesis in the rumen, which could have been limited by N and/or sulphur availability. Egan (1977) and Doyle and McLaren (1988) hypothesized that protein supply to the tissues either in relation to digestible energy intake or in absolute terms may limit intake of low-quality forages.

The higher ammonia N and VFA concentration in the rumen of animals supplemented with urea resulted in a higher osmolality. The osmolality of blood is around 280 milli-osmoles (Nagabushanam et al., 1983). The rumen fluids at the lowest supplementation levels were hypotonic to blood, probably because of the high influx of water through the infusate, which probably exceeded the outflow and absorption of water from the rumen.

It can be concluded that urea supplementation increased intake in association with a higher rate of passage of the particulate phase without affecting digestion. The digestible organic matter intake was below maintenance for all supplementation levels. Assuming maintenance requirements of 26 g DOMI/$kg^{0.75}$/day for goats (adapted from Agricultural Research Council, 1980) only 49 % of the maintenance requirements were met by unsupplemented wheat straw and 62 % by urea supplemented wheat straw.

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CHAPTER 3.

AMMONIA TREATMENT OF WHEAT STRAW

3.1 Ammonia treatment of wheat straw. 1. Voluntary intake, chewing behaviour, rumen pool size and partition of digestion along the gastro-intestinal tract of sheep

3.2 Ammonia treatment of wheat straw. 2. Efficiency of microbial protein synthesis, rumen microbial protein pool size and turnover, and small intestinal protein digestion in sheep

3.3 Intake and utilization of energy from ammonia-treated and untreated wheat straw by steers and wether sheep fed a basal diet of grass pellets and hay

3.1 Ammonia treatment of wheat straw. 1. Voluntary intake, chewing behaviour, rumen pool size and turnover and partition of digestion along the gastro-intestinal tract of sheep

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Abstract

The impact of ammonia treatment of wheat straw on intake and digestion and passage kinetics was studied with 6 wether sheep. Ammoniated wheat straw (AWS) was compared with untreated wheat straw (UWS) and untreated wheat straw supplemented with urea (SWS). Ammonia treatment increased intake and whole tract digestion without affecting rumen pool size and rate of passage significantly. The increased rumen cell wall turnover due to ammonia treatment could for 91 % be attributed to a higher rumen degradation of cell walls. No significant effects of ammonia treatment on concentration, molar composition or rate of absorption of volatile fatty acids were observed. The rate of passage in the hindgut and the contribution of the hindgut to whole tract digestion were not significantly affected by ammonia treatment. Rumination and eating time and daily number of rumen contractions did not differ between rations, but AWS showed a lower rumination time per kg NDF ingested than the control rations. Whole tract digestion and rumen degradation of hemicellulose increased more due to ammonia treatment than those of cellulose. The effects of ammonia treatment could not be attributed to N supplementation, since no effect of urea supplementation on any parameter, except rumen ammonia-N concentration was observed.

Keywords: wheat straw, ammonia treatment, site of digestion, rumination, rumen degradation, rumen passage, hindgut passage.

Introduction

In small-scale farming systems in densely populated areas in the tropics the productivity of the ruminant livestock depends to a large extent on fibrous crop residues like rice and wheat straw. These crop residues are generally characterized by a low intake and digestibility. Improvement of the nutritional quality of fibrous crop residues may be achieved through selection and breeding of varieties with a relatively good straw quality (Capper, 1988), supplementation with deficient nutrients and alkaline treatment (Sundstøl & Owen, 1984). Alkaline treatment of straw with ammonia or NaOH as the reagent, improves microbial digestion of the cell wall fraction through disruption of ester bonds between hemicellulose and lignin (Mason et al., 1990; Morrisson, 1983; Chesson et al., 1983).

The extent of microbial digestion in the reticulo-rumen is determined by the rate and potential extent of degradation of digesta and the retention time of particles in the reticulo-rumen. Retention time in the reticulo-rumen is related to particle size distribution (Poppi et al., 1980; Egan & Doyle, 1984) and functional specific gravity of particles (Sutherland, 1987). The contribution of microbial degradation to particle size reduction is limited and reduction of particle size is therefore mainly determined by chewing during eating and rumination (Ulyatt et al., 1986).

Particle size reduction is, however, probably not the main rate limiting step for passage of particles from the reticulo-rumen (Ulyatt et al., 1986). Passage of particles is higher during eating than during resting and rumination (Girard, 1990), which could be related to the higher frequency of rumen contractions during eating (Ulyatt et al., 1986). Okine et al. (1990) observed, that the duration of individual rumen contractions rather than frequency of rumen contractions was positively related to geometric mean particle size of faeces, indicating an increased probability of passage of larger particles with increased duration of rumen contractions.

Increased intake is often associated with increased rate of passage of particles, but ruminants with higher intake may also increase the rumen load of particles either by increasing the total rumen load or by increasing the DM content of the rumen digesta (Owens & Goetsch, 1986, Bosch et al., 1992).

Increased particle passage from the rumen may result in a lower degradation, particularly due to a reduced duration of microbial degradation. This may, however, in part be compensated by a higher hindgut fermentation, since more potential degradable material becomes available for microbial degradation in the caecum and colon (Demeyer, 1991).

The nutritive value of low quality feeds is in addition to voluntary intake and digestion related to proportions of individual volatile fatty acids produced and the amount of amino acids that become available for absorption from the small intestine. Propionic acid is a precursor for gluconeogenesis, while acetate and butyrate can only be utilized for energetic purposes and fat synthesis. In case of limited propionate production amino acids could be utilized for gluconeogenesis. The amount of amino acids that come available for absorption in the small intestine depends, in case of low quality feeds largely on the quantity of

microbial protein synthesized in the rumen (Hvelplund, 1989).

The experiment reported here had as objective to study the implications of ammonia treatment of wheat straw on parameters as

- voluntary intake, digestion and site of digestion
- eating and rumination behaviour
- rumen pool size
- rumen degradation and rumen and hindgut passage.

The effect of ammonia treatment on amino acid and N digestion is reported elsewhere (Oosting et al., 1993).

Materials and methods.

Six sheep (wethers) with an average live weight of 44 kg were fitted with a rumen cannula (Bar Diamond Inc. 3 inch diameter) in the dorsal rumen sac, a silastic infusion tube (3 mm i.d.) in the abomasum and with T-shape hard pvc cannulas (12 mm i.d.) in the proximal duodenum and terminal ileum. The experiment started 2.5 months after surgery, after the animals had well recovered.

During the experiment the animals were kept in metabolic cages and received equal portions of their ration at 04.00, 08.00, 12.00, 16.00, 20.00 and 24.00 h. Water and a mineral lick containing NaCl, Fe, Mg, Mn and Co were freely available.

The animals were randomly allotted into three groups of two sheep each. Group one was fed untreated wheat straw *ad libitum* supplemented with pelleted sugar beet pulp (UWS). Group two was fed the same ration with an infusate into the rumen of an urea solution (60 ml/h; concentration of 5.4 g urea-N/l; 7.9 g N daily) (SWS) and group three was fed ammoniated wheat straw *ad libitum* also with a supplement of pelleted sugar beet pulp (AWS).

The untreated and ammoniated wheat straws were offered in a quantity of 1.8 kg daily, thus allowing selection. The sugar beet pulp was supplied at a level of 240 g product daily. The composition of the feeds is given in Table 1.

The wheat straw was ammoniated in the late summer of 1989 by injecting 40 kg of anhydrous ammonia into stacks of 900 kg baled straw, totally covered with two layers of polythene sheets of 0.15 mm thickness. During injection, tubes were inserted in the bottom of the stacks to let the air out. After injection these tubes were removed. The stacks were opened after 35 days.

Table 1. Composition (g/kg) of untreated wheat straw (UWS), ammoniated wheat straw (AWS) and pelleted sugar beet pulp (SBP).

	UWS	AWS	SBP
DM	887	843	886
Ash[1]	84	85	105
N[1]	6	18	13
NDF[1]	785	751	425
Hemicellulose[1]	285	250	151
Cellulose[1]	425	437	237
Lignin[1]	75	64	37

[1] In DM

Before the onset of the experiment the animals were well adapted to the straw rations and the whole experimental routine. Before the start of the experiment untreated straw was fed during 5 weeks. Subsequently, the experiment consisted of a two week adaptation period and a seven week experimental period, in which the various measurements were conducted as indicated in Table 2.

In experimental weeks 1 and 6, the turnover rates of the liquid and particulate phases in the reticulo-rumen were estimated with Co-EDTA and Cr-NDF (Udén et al., 1980), respectively. On Monday at 08.00 h, 3.4 g Co-EDTA (0.51 g Co) and 10 g Cr-NDF (0.43 g Cr, particle size 0.2-1.0 mm) were introduced into the ventral rumen sac. Subsequently, rumen fluid samples were collected at 09.00, 10.00, 11.00, 12.00, 14.00, 16.00, 18.00, 20.00, 22.00 h on Monday and at 0.00, 2.00, 4.00, 6.00, 8.00, 12.00, 16.00 and 20.00 h on Tuesday. Co was determined with an atomic absorption spectrophotometer (Varian SpectraA 300; 240.7 nm) after wet destruction.

Total collection of faecal excreta was done from Tuesday 08.00 h to Thursday 0.00 h every 4 hours, from Thursday 0.00 h till Saturday 08.00 h every 8 hours and from Saturday 08.00 h till Sunday 20.00 h every 12 hours. After subsampling, faeces were dried and Co and Cr were measured after wet destruction by atomic absorption spectrophotometry at wavelengths of 240.7 and 357.9 nm, respectively.

The dilution rate of the liquid phase marker (Co) was estimated directly in the rumen fluid ($k_{l\text{-rumen}}$) and from the faecal excretion pattern ($k_{l\text{-faeces}}$, adapted from Grovum & Williams, 1973). The fractional passage of the particulate phase marker (Cr) from the reticulo-rumen was derived from the descending part of the faecal excretion curve (k_p, adapted from Grovum & Williams, 1973). The reticulo-rumen liquid volume was estimated by extrapolation of the Co dilution curve obtained from direct sampling of rumen fluid to t

= 0.

The pH, ammonia and volatile fatty acid (VFA) concentrations in the rumen fluid were measured in samples taken on Monday, hourly from 09.00 till 12.00 h, and two hourly from Monday 12.00 h till Tuesday 08.00 h in experimental weeks 1 and 6. The ammonia concentration was measured by the indophenol method (Scheiner, 1976). The extinction of the blue colour was measured at 634.8 nm with a Hitachi U 2000 spectrophotometer. VFA concentrations were determined by gas liquid chromatography (Packard 419, glass column filled with chromosorb 101, carrier gas N_2 saturated with formic acid, 190 °C with isocapronic acid as an internal standard).

The kinetics of fermentative degradation of untreated and ammoniated wheat straw were studied *in sacco* starting on Friday before experimental weeks one and six. Three gram of wheat straw or ammoniated wheat straw (ground to pass a 1 mm sieve) were weighed into dacron bags (size 70x120 mm; pore size 40 μm). The bags were incubated over 0.5, 6, 12, 24, 36, 48 and 72-h periods. Each straw was incubated in the rumen of a sheep consuming the same experimental straw. For the estimation of the truly undegradable fraction, the straws were incubated at the end of the experiment for 240 hours. After removal from the rumen and washing with tap water till the effluent was clear, the bags were dried at 70°C during 24 hours, after which the residue was analyzed for DM, ash and NDF. The degradation parameters were estimated according to the following model (Robinson et al., 1986):

$$f_t = f_r + f_d * e^{-kd*(t-t')} \qquad (1)$$

in which f_t represents the residual fraction of a feed component at time = t, f_d the potentially degradable fraction, f_r the truly undegradable fraction, k_d the fractional rate of degradation and t' the lag-time before microbial digestion starts. Fitting of the model was done by the non-linear regression option of the statistical program DBSTAT (Brouwer, 1989).

Rumen contractions and eating and rumination activity were measured in weeks 1 and 6. Rumen contractions were recorded with an open tip catheter connected to a pressure transducer. Specifications were as described by Bosch et al. (1988). Chewing activity was measured by a switch attached with a halter to the lower jaw of the animals.

Table 2. Time chart of activities over the experimental period.

	experimental week[1]						
	1111111	2222222	3333333	4444444	5555555	6666666	7777777
In sacco degradation	** *				**	*	
Rumen NH_3/VFA/pH	*					*	
Eating/rumination	******					******	
Rumen passage	******	*				******	*
Rumen contractions	******					******	
Hindgut passage		***			***		
Flow small intestine			****	****			
Intake and digestion			******	******			
Rumen evacuation							***

[1] days within week sunday up to saturday.

In experimental weeks 2 and 5, the passage of liquid and particles from the hindgut was measured by administering into the ileum about 50 g of a aquous solution/suspension containing 4.0 % Cr-NDF (4.3 % Cr), 1.5 % Carboxy Methyl Cellulose, 0.9 % NaCl and 1.3 % Co-EDTA (14.9 % Co) at 20.00 h on Wednesday. Subsequently, total faecal excreta were collected at moment of defaecation on Thursday and Friday from 06.00 till 18.00 h and analyzed for Co and Cr as described earlier. The rate of passage of the markers was estimated from the model:

$$C_t = C_0 * e^{-k*t} \qquad (2),$$

in which C_t is the concentration of the marker in the faeces at time = t and k is either the rate constant of passage of Cr (k_p) or of Co (k_l).

In the 3rd and 4th week of the experiment the flow of nutrients in the duodenum and ileum was assessed based on a continuous infusion of about 20 mg Co and 20 mg Cr per hour into the abomasum, starting one day prior to sampling. The infusate had the following composition: 0.75 % Cr-NDF (4.3 % Cr), 0.26 % Co-EDTA (14.9 % Co), 0.9 % NaCl, 1.5 % Carboxy Methyl Cellulose and 96.6 % water. Hourly samples (about 10 g) of duodenal and ileal digesta were collected from Monday till Friday from 08.00 till 20.00 h. The samples were freeze dried, ground and pooled per sheep per week and cannula, and subsequently analyzed for dry matter (DM), ash, Co and Cr, neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Duodenal and ileal collection was done simultaneously. Duodenal or ileal flow of a constituent was estimated from its concentration relative to the concentration of the markers. Correction of the ileal flow for withdrawal of digesta in the duodenum was considered not necessary because the concentration of a constituent relative to the concentration of markers in ileal digesta is not affected by duodenal sampling.

Digestion was measured by daily collection and subsampling of residual feed and total collection of faeces over six days in weeks 3 and 4. Faecal excretion was not corrected for duodenal and ileal sampling, which was done during four of the six days of faecal collection. The average DM withdrawal over the faecal collection period by duodenal and ileal sampling was approximately 10 g/d. Since part of the withdrawn DM would be degraded in the large intestine, the actual underestimation of faecal DM excretion was less than 10 g/d.

During the 7th week of the experiment total rumen evacuations were conducted in such a schedule that of all animals total rumen contents were measured and a proportional sample for analyses of DM, ash, NDF, ADF and ADL and sieve analysis was taken at 1, 2 and 3 hours after feeding. Analyses were done in samples pooled per animal. Wet sieve analysis of rumen samples of approximately 50 g was done on a Fritsch Analysette 3 over a sieve of 1.25 mm. After sieving the material retained on the sieve was quantitatively collected and dried at 103^{0}C.

Prior to the experiment described above an intake and digestion experiment was conducted with all six sheep fed untreated wheat straw. The experimental period lasted six days with an adaptation period of two weeks. During this experiment one dacron bag with untreated wheat straw was incubated in the rumen of each sheep for 48 hours. The procedure was equal to the procedure described before. Sugar beet pulp was incubated for 48 hours in duplicate in the rumen of two spare sheep fed untreated wheat straw .

DM and ash were determined by drying at 103 °C and ashing at 550 °C, respectively. N was determined by the Kjeldahl method with K_2SO_4 and $CuSO_4$ as catalysts. NDF, ADF and ADL were determined according to Goering & Van Soest (1970). Hemicellulose was calculated as NDF - ADF and cellulose as ADF - ADL.

Statistical analysis was done on means per animal (average of two repeated measurements) by the program DBSTAT (Brouwer, 1989). The model was: Y_{ij} = mean + $treatment_i$ + $error_{ij}$, with total degrees of freedom (d.f.) 6. To test the significance of contrasts: L1 : -1*UWS - 1*SWS + 2*AWS and L2 : -1*UWS + 1*SWS, the treatment sum of square was subdivided in the sum of square of each contrast with d.f. = 1. The d.f. of the error term was 3. (Snedecor & Cochran, 1967).
The level of significance of a contrast was indicated in tables for $p < 0.25$ (§), $p < 0.10$ (†), $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

For testing of differences between parameters the difference per animal was calculated and analyzed by the full model. Whether the overall mean difference or the difference within rations differed significantly from zero was tested by Student's t-test (Snedecor & Cochran, 1967).

Results

Voluntary intake, digestion and site of digestion.

For calculation of the organic matter digestibility (OMD) of straw the OMD of sugar beet pulp was assumed 850 g/kg. The *in sacco* OMD of sugar beet pulp after 48 h incubation in the rumen of two sheep fed untreated wheat straw was 948 g/kg. Intake (I) of DM, OM and digestible organic matter (DOM) of the whole ration as well as of the straw component of the ration was significantly increased by ammonia treatment (Table 3). DMD and OMD of the whole ration tended to increase due to ammonia treatment, while DMD and OMD of the straw component of the rations were significantly higher for AWS than for UWS and SWS. Urea infusion had no effect on intake and digestion of DM and OM. Ammoniation of wheat straw had more effect on intake than on digestibility of wheat straw. Ammoniation of wheat straw increased OMI of straw by 87 %, OMD of straw by 43 %, while DOMI of straw increased by 180 %.

Assuming maintenance requirements of 26 g DOMI/$kg^{0.75}$/d for sheep (ARC, 1980) the rations UWS, SWS and AWS could cover 70, 75 and 133 % of the maintenance requirements, respectively.

Intake and digestion of cell wall components of whole rations were higher for AWS than for UWS and SWS (Table 4), though the effects of ammonia treatment on digestion of cellulose and lignin were not significant ($p > 0.05$). Hemicellulose digestion increased more due to ammonia treatment than cellulose digestion (41 % and 15 %, respectively). Urea infusion had no significant effect on intake and digestion of cell wall components.

Table 3. Intake and digestion of DM and OM, DOMI and average weight of the animals.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
Intake (g/$kg^{0.75}$/d)						
DM - *whole ration*	36.0	39.2	59.0	*	ns	3.27
- *straw*	23.3	26.6	46.8	*	ns	3.34
OM - *whole ration*	32.6	35.6	53.6	*	ns	2.98
- *straw*	21.3	24.4	42.7	*	ns	3.05
Digestibility (g/kg)						
DM - *whole ration*	544	519	611	†	ns	24.7
- *straw*	372	360	548	*	ns	21.9
OM - *whole ration*	569	545	641	†	ns	27.1
- *straw*	416	403	587	*	ns	23.1
DOMI (g/$kg^{0.75}$/d)						
- *whole ration*	18.3	19.4	34.5	**	ns	1.48
- *straw*	8.3	9.4	24.8	**	ns	1.63
Average weight of animals (kg)	43.1	43.3	45.5	§	ns	1.05

ns: not significant, §: $p < 0.25$, †: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

Table 4. Intake (I) and digestion (D) of cell wall components of whole rations.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
NDF						
I ($g/kg^{0.75}/d$)	24.3	27.2	40.7	*	ns	2.77
D (g/kg)	572	544	695	*	ns	29.3
Hemicellulose						
I ($g/kg^{0.75}/d$)	7.7	9.3	13.4	*	ns	1.04
D (g/kg)	540	523	749	**	ns	29.3
Cellulose						
I ($g/kg^{0.75}/d$)	14.5	15.6	23.5	*	ns	1.54
D (g/kg)	656	618	730	†	ns	24.4
Lignin						
I ($g/kg^{0.75}/d$)	2.2	2.4	3.5	*	ns	0.23
D (g/kg)	112	141	243	§	ns	58.7

ns: not significant, §: $p < 0.25$, †: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

Selective consumption of straw was not observed for AWS. In UWS and SWS a positive selection of cellulose occurred. The cellulose content of the straw part of UWS and SWS offered was 425 g/kg and of the straw consumed 486 g/kg ($p < 0.05$). Insignificant differences existed between the NDF and hemicellulose contents of the straw part of UWS and SWS offered and consumed. In the straw offered NDF was 785 g/kg and hemicellulose 286 g/kg, while in the straw consumed NDF was 817 g/kg and hemicellulose 264 g/kg. The animals probably selected for leaves, which contain more cellulose and less hemicellulose than stems (Wales et al., 1990). The ratio of Co to Cr in the marker mixture infused for estimation of flows of digesta in the duodenum and ileum was 1.16. In the duodenal samples this ratio was 1.17 (SEM 0.04) and in ileal samples 1.14 (SEM 0.04), indicating, that representative samples of duodenal and ileal digesta with respect to fluid and particulate phases were obtained.

Based on estimates of flows of nutrients at various sites of the digestive tract, the contribution of digestion in a particular site of the digestive tract to whole tract digestion was calculated. Estimates of this partial digestion in the rumen, small intestine and large intestine are presented in Table 5. No significant differences emerged between rations in respect of the contributions of various sites of the digestive tract to whole tract digestion. Partial digestion in the rumen was significantly lower and partial digestion in the large intestine significantly higher for OM than for cell wall components. The contribution of the small intestine to whole tract digestion of OM and cell wall components was low and did over and

Table 5. Partial digestion in various sites of the digestive tract (% of whole tract digestion)

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
OM - *rumen*	75.8	79.9	81.8	ns	ns	3.64
- *small intestine*	1.4	-1.5	6.0	§	ns	2.87
- *large intestine*	22.8	21.6	12.2	§	ns	5.74
NDF - *rumen*	94.1	92.6	96.3	ns	ns	5.43
- *small intestine*	-5.1	-2.0	-1.3	ns	ns	1.79
- *large intestine*	10.9	9.4	5.0	ns	ns	6.93
Hemicellulose						
- *rumen*	92.6	92.4	97.6	ns	ns	4.24
- *small intestine*	-5.0	-1.6	-0.4	§	§	1.50
- *large intestine*	12.4	7.6	2.8	ns	ns	5.55
Cellulose						
- *rumen*	86.1	86.6	90.0	ns	ns	3.88
- *small intestine*	0.1	-0.2	1.0	ns	ns	2.32
- *large intestine*	13.8	13.6	9.1	ns	ns	4.50

ns: not significant, §: $p < 0.25$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

within rations not differ significantly from zero. The apparent digestion of lignin occurred in the rumen, where on average 383 g/kg lignin intake was degraded. In the small and large intestines a negative digestion of lignin was observed of respectively 94 and 115 g/kg lignin intake. The latter values did not differ significantly from zero.

The flows of potentially degradable NDF (NDF_d) at various sites of the digestive tract are given in Table 6. Assuming an f_r of sugar beet pulp of 100 g/kg NDF and of straw NDF equal to $f_r/(f_r + f_d)$ (see Table 9) the intake of truly undegradable NDF (NDF_r) could be estimated. In a steady state situation, the amount of NDF_r entering a digestion compartment is equal to the amount flowing out of that compartment. The flows of NDF_d at the various sites of the digestive tract could therefore be calculated as the difference between the total NDF flow minus intake of NDF_r.

No significant differences emerged between rations with regard to intake of NDF_r and flows of NDF_d in duodenum, ileum or faeces. Faecal excretion of NDF_d was slightly underestimated due to withdrawal of digesta from duodenum and ileum. NDF withdrawal from duodenum and ileum was approximately 5 g/d averaged over the faecal collection period.

Table 6. Flows of truly indigestible NDF (NDF_r) and potentially degradable NDF (NDF_d) and degradation of NDF_d in various sites of the digestive tract.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
Intake (g/d)						
- NDF_r	123	140	111	ns	ns	17.6
- NDF_d	287	319	596	**	ns	35.6
Duodenal flow (g/d)						
- NDF_d	70	90	123	§	ns	15.8
Ileal flow (g/d)						
- NDF_d	81	96	130	§	ns	18.9
Faecal excretion (g/d)						
- NDF_d	57	70	103	§	ns	16.5
Degradation of NDF_d (g/kg flow)						
- *whole tract*	809	781	824	ns	ns	30.0
- *rumen*	758	723	793	§	ns	28.7
- *large intestine*	317	205	200	ns	ns	90.8

ns: not significant, §: $p < 0.25$, ** $p < 0.01$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

Whole tract degradation of NDF_d was over rations 805 g/kg NDF_d ingested. The average degradation of NDF_d in the rumen was 758 g/kg NDF_d ingested and in the large intestine 241 g/kg NDF_d entering the small large intestine. The latter value would be slightly lower, if a correction was made for the underestimation of faecal NDF_d excretion.

The degradation of NDF_d in a digestion compartment can be described by $k_d/(k_d + k_p)$ (Aitchisson et al., 1986). The k_p of Cr-NDF in the large intestine was on average 10.7 %/h (Table 10). Assuming that this was also the k_p of NDF_d in the large intestine, the k_d of NDF_d was over rations estimated as 4.8 %/h (s.e. of estimate 1.95, no significant differences between rations).

Passage of NDF from the rumen could be calculated as duodenal NDF flow/rumen NDF pool ($k_{p,eff}$, see Table 11). On the assumption, that the $k_{p,eff}$ of NDF_d was equal to the $k_{p,eff}$ of NDF, the estimated k_d of NDF_d was 5.3, 4.2 and 6.1 %/h (SEM 0.99) for UWS, SWS and AWS, respectively. These estimates did not differ significantly between rations, but differed significantly from the values given in Table 9.

Eating and rumination behaviour

An overview of the results obtained from measurements of ingestion and rumination characteristics and of rumen contractions is given in Table 7. No significant differences were observed in total daily chewing time, eating time and rumination time between the rations. However, per kg NDFI less time was spent chewing ($p < 0.05$), ruminating ($p < 0.001$) and eating ($p < 0.25$) for AWS relative to UWS and SWS. The number of chews per minute eating or rumination was not different between rations as was the number of boli per minute rumination. The frequency of chewing during eating was higher than during rumination.

No differences were observed in rumen contractions between the rations, neither in total daily number, nor in frequency during eating, rumination or resting. Over rations the frequency of rumen contractions was higher during eating than during resting and rumination ($p < 0.05$).

Rumen pool size

Table 8 presents the rumen pool sizes for the three rations tested. The within ration variation was high and consequently no significant differences between rations could be observed. In line with the lower chewing activity per kg NDF ingested, a significantly higher proportion of large particles was found in the rumen DM of sheep fed with ammoniated wheat straw. The DM contents of rumen digesta were 122, 126 and 111 g/kg (SEM 4.1) for UWS, SWS and AWS, respectively. The DM content was lower for AWS than for UWS/SWS ($p < 0.05$).

Table 7. Daily chewing time (CT), eating time (ET) and rumination time (RT), related chewing characteristics and number and frequency of rumen contractions

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
CT (min/day)	735	826	816	ns	ns	59.8
ET (min/day)	210	233	209	ns	ns	36.0
RT (min/day)	526	593	604	ns	ns	59.9
CT (min/kg NDFI)	1813	1815	1148	*	ns	122.3
ET (min/kg NDFI)	534	519	292	§	ns	108.7
RT (min/kg NDFI)	1279	1297	856	***	ns	34.9
Frequency of chewing (chews/min)						
-during eating	117	104	114	ns	ns	18.5
-during rumination	93	89	97	ns	ns	3.6
Rumination boli (per minute RT)	0.92	1.04	1.06	ns	§	0.053
Rumen contractions						
-total daily	2790	2733	2771	ns	ns	116.0
-frequency (number/min)						
- *eating*	2.8	2.8	2.7	ns	ns	0.13
- *rumination*	1.7	2.0	1.8	ns	ns	0.12
- *resting*	1.9	1.5	1.8	ns	ns	0.19

ns: not significant, §: $p < 0.25$, *: $p < 0.05$, ***: $p < 0.001$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

Table 8. Rumen pool size and distribution of particle size.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
Total pool size						
- (g)	6394	7348	9430	§	ns	1481.8
- (% of weight)	15.4	17.7	21.2	ns	ns	2.94
DM (g)	775	917	1054	ns	ns	182.3
OM (g)	737	877	996	ns	ns	175.0
NDF (g)	529	617	628	ns	ns	129.8
Hemicellulose (g)	210	244	217	ns	ns	50.2
Cellulose (g)	266	306	341	ns	ns	63.6
Lignin (g)	52	67	70	ns	ns	16.5
Particles > 1.25 mm (% of DM)	14.9	15.8	21.0	*	ns	1.21

ns: not significant, §: $p < 0.25$, *: $p < 0.05$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

Rumen degradation and rumen and hindgut passage.

Degradation characteristics of OM and NDF as determined by dacron bag incubations are presented in Table 9. Ammonia treatment increased the potentially degradable part of OM and NDF significantly and reduced the truly undegradable part significantly. However, no significant effect of ammonia treatment on the rate of degradation was observed. Differences in degradation between untreated wheat straw incubated in the rumen of sheep fed UWS or SWS were not observed.

Table 9. Truly undegradable fraction (f_r), potentially degradable fraction (f_d), rate of degradation (k_d) and lag-time (t') of straw OM and NDF.

		UWS	SWS	AWS	Significance of contrast		SEM
					L1	L2	
OM:	f_r (%)	33.3	33.3	17.3	***	ns	0.25
	f_d (%)	51.6	52.0	66.7	***	ns	0.52
	k_d (%/h)	1.85	1.92	2.23	§	ns	0.192
	t' (h)	3.4	4.2	2.8	§	ns	0.53
NDF:	f_r (%)	34.1	34.1	15.6	***	ns	0.23
	f_d (%)	61.7	62.0	79.4	***	ns	0.50
	k_d (%/h)	1.98	2.07	2.55	§	ns	0.211
	t' (h)	3.7	4.1	2.5	§	ns	0.71

ns: not significant, §: $p < 0.25$, ***: $p < 0.001$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

Table 10. Fractional rate of passage of the liquid (k_l) and particulate (k_p) phase to the lower gut and from the hindgut and rumen liquid volumes.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
Reticulo-rumen						
$k_{l\text{-rumen}}$ (%/h)	6.3	5.7	7.2	ns	ns	0.82
$k_{l\text{-faeces}}$ (%/h)	4.3	4.0	3.9	ns	ns	0.42
k_p (%/h)	2.7	2.4	2.8	ns	ns	0.46
Hindgut						
k_l (%/h)	10.2	9.2	10.4	ns	§	0.44
k_p (%/h)	11.5	10.5	10.2	§	†	0.30
Rumen liquid volume						
- *direct (l)*	5.6	6.3	8.8	§	ns	1.21
- *CoEDTA (l)*	4.8	6.5	7.2	ns	ns	0.98

ns: not significant, §: $p < 0.25$, †: $p < 0.10$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

No significant differences in fractional outflow rates as measured by markers from the rumen or hindgut were found between rations (Table 10). Over rations estimates of $k_{l\text{-rumen}}$

were higher than estimates of $k_{i\text{-faeces}}$ ($p < 0.05$). In contrast to the rumen, in the hindgut k_i and k_p did not differ. The faecal excretion pattern of Cr and Co administered into the ileum could nicely be described by the first order model. R^2 values for these excretion curves, but also for dilution curves of Co in the rumen and faecal excretion curves of Co and Cr administered into the rumen were all higher than 0.97.

Rumen liquid volumes as derived from direct measurements (rumen evacuations) and rumen Co-EDTA dilution are also given in Table 10. No significant differences were found between both methods of estimation.

Table 11 presents the rumen turnover (k_{cl} (%/h) = intake/rumen pool size), the effective passage ($k_{p,eff}$ (%/h) = duodenal flow/rumen pool size) and the effective degradation ($k_{d,eff}$ (%/h) = (intake - duodenal flow)/rumen pool size) for cell wall components.

Table 11. Rumen clearance (k_{cl} = intake/rumen pool/24, %/h), effective rumen passage ($k_{p,eff}$ = duodenal flow/rumen pool/24, %/h) and effective rumen degradation ($k_{d,eff}$ = (intake - duodenal flow)/rumen pool/24, %/h).

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
NDF: k_{cl}	3.4	3.2	4.8	†	ns	0.45
$k_{p,eff.}$	1.5	1.6	1.6	ns	ns	0.19
$k_{d,eff}$	1.9	1.6	3.1	*	ns	0.28
Hemicellulose:						
k_{cl}	2.7	2.7	4.6	*	ns	0.34
$k_{p,eff.}$	1.4	1.3	1.2	ns	ns	0.16
$k_{d,eff}$	1 3	1.3	3.3	**	ns	0.19
Cellulose						
k_{cl}	4.1	3.6	5.1	§	ns	0.51
$k_{p,eff.}$	1.8	1.7	1.7	ns	ns	0.18
$k_{d,eff}$	2.3	2.0	3.4	†	ns	0.35
Lignin						
k_{cl}	3.1	2.5	3.8	ns	ns	0.59
$k_{p,eff.}$	2.5	1.9	2.5	ns	ns	0.51
$k_{d,eff}$	0.7	0.6	1.3	†	ns	0.19

ns: not significant, §: $p < 0.25$, †: $p < 0.10$, * $p < 0.05$, ** $p < 0.01$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

The k_{cl} increased with ammoniation, but the increase was only significant for hemicellulose. The $k_{p,eff}$ did not differ between rations. Over rations, hemicellulose had a significantly lower $k_{p,eff}$ than cellulose, while lignin had a significantly higher $k_{p,eff}$ than the other cell wall components.

The $k_{d,eff}$ of cell wall components was higher for AWS than for UWS and SWS, which was significant for NDF and hemicellulose. The $k_{d,eff}$ of hemicellulose was significantly lower than that of cellulose for UWS and SWS, but not for AWS.

In Table 12, the average daily rumen pH, ammonia-N concentration and concentration, profile and rate of absorption of VFA's are presented. The diurnal variation in these parameters was low. The VFA production was calculated from the apparently rumen degraded OMI (ARDOMI = OMI - OM flow in the duodenum) by assuming a molecular weight of glucose in polymerized carbohydrates of 162 g/mole and a VFA production of 100/(0.5*(proportion of HAC + proportion of HPr) + proportion of HBu) per mole glucose (Czerkawski, 1986). The turnover was estimated as the hourly VFA production/VFA pool in the reticulo-rumen, whereas the rate of absorption of VFA was derived from VFA turnover - $k_{t\text{-rumen}}$.

No differences between rations were observed in average daily pH, VFA concentration and profile of VFA, non glucogenic/glucogenic ratio (NGR) and VFA turnover

Table 12. Rumen concentration of VFA and NH_3-N, pH and rate of turnover and rate of absorption of VFA's in the rumen.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
pH[1]	6.81	6.84	6.84	ns	ns	0.097
NH_3-N[1] (mg/l)	42.6	225.4	175.2	†	**	12.28
VFA[1] (mmol/l)	107.1	91.9	111.6	ns	ns	15.94
Profile (mol/100 mol)						
HAc	70.7	73.0	70.7	ns	ns	1.18
HPr	19.1	17.3	18.3	ns	*	0.29
HBu	9.7	9.3	10.0	ns	ns	0.74
HVa	0.5	0.4	1.0	ns	ns	0.27
NGR[2]	4.6	5.2	4.8	ns	*	0.12
VFA turnover[3] (%/h)	21.8	22.3	23.7	ns	ns	6.25
VFA absorption[4] (%/h)	15.5	16.6	16.5	ns	ns	6.01

ns: not significant, †: $p < 0.10$, *: $p < 0.05$, ** $p < 0.01$
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.
[1] daily average
[2] Non glucogenic/glucogenic-ratio; (HAc+2*HBu+HVa)/(HPr+HVa)
[3] VFA production/VFA pool/24. VFA production = ARDOMI/162 * (100/(0.5*(proportion HAc + proportion HPr) + proportion HBu).
[4] VFA turnover - $k_{t\text{-rumen}}$.

and absorption rate of VFA, except for the contrast UWS versus SWS in case of molar proportion of HPr in the VFA pool and consequently NGR. UWS showed a significantly lower ammonia N concentration than SWS and AWS. Over rations, absorption of VFA's contributed for 72 % to the rumen turnover of VFA's.

Discussion

Intake, digestion and site of digestion

Voluntary intake and digestion of wheat straw increased due to ammonia treatment. The increase in straw OMI in the present experiment was higher than that observed by Silva et al. (1989) for barley straw in sheep and by Dias-da-Silva & Sundstøl (1986) for wheat straw in sheep. In these experiments, however, the intake of untreated straw was higher than that in the present experiment. The increase in OMD of wheat straw due to ammoniation was comparable with that found by Dias-da-Silva & Sundstøl (1986). The effect of ammonia treatment on intake was higher than the effect on digestibility.

The effect of ammonia treatment on intake and digestion of straws is attributed to cleavage of ester linkages between hemicellulose and lignin (Morrisson, 1983; Chesson et al., 1983; Mason et al., 1990). After treatment generally an increased digestibility of hemicellulose is observed, but also cellulose digestion may increase, sometimes as much as that of hemicellulose (Lindberg et al., 1984), probably due to enhanced microbial activity (Chesson et al., 1983). In the present experiment, *in vivo* digestion and effective rumen degradation of hemicellulose increased more after ammonia treatment than those of cellulose. A higher effective rumen degradation could either be attributed to a higher potentially degradable fraction and/or to an increased rate of degradation of the potentially degradable fraction.

The effect of sugar beet pulp supplementation on intake and digestion of untreated wheat straw is summarized in Table 13. OMI and OMD of UWS without sugar beet pulp supplementation were measured in the experiment conducted prior to the main experiment. Results for UWS and SWS from the main experiment were combined and compared with results for the same sheep in the earlier experiment.

Supplementation of UWS/SWS with 11.3 g OM/$kg^{0.75}$/d from sugar beet pulp reduced straw OMI with 8.9 g/$kg^{0.75}$/d. Both *in vivo* and *in sacco* OMD of UWS decreased due to sugar beet pulp supplementation. If it was assumed, that the truly undegradable and the soluble fractions and the lag time of UWS were not affected by sugar beet pulp supplementation, for UWS incubated in the rumen of sheep fed unsupplemented UWS the rate of degradation of OM could be calculated from the OM disappearance after 48 h. The derived estimate was 2.52 %/h compared to the value of 1.88 %/h for UWS incubated in the rumen of sheep fed UWS/SWS supplemented with sugar beet pulp. This reduction in rate of

Table 13. Intake and digestion parameters of UWS supplemented (+) or unsupplemented (-) with sugar beet pulp (between brackets SEM). Data for the four sheep fed UWS or SWS during the main experiment.

Supplementation	-	+
OMI (g/kg$^{0.75}$/d)		
- sugar beet pulp	0	11.3
- UWS/SWS	31.7^{b} (0.20)	22.8^{a} (1.99)
OMD (g/kg)		
- *in vivo*	499^{b} (16.5)	410^{a} (17.3)
- *in sacco (48 h)*	497^{b} (6.3)	457^{a} (9.0)

Different superscripts per row indicate significant differences ($p < 0.05$).

degradation of OM could, at least partly, explain the reduction in intake of OM from UWS due to sugar beet pulp supplementation. The pH in the rumen of sheep fed UWS/SWS supplemented with sugar beet pulp was 6.8 with almost no diurnal variation. It is therefore unlikely, that the effect of sugar beet pulp on intake and digestion of wheat straw could be attributed to a decreased pH.

In contrast to the results given above, Silva et al. (1989) observed positive effects of sugar beet pulp supplementation on intake and digestion of untreated or ammoniated barley straw fed to sheep and cattle. In the present experiment, however, the supplementation level was higher than in the experiment of Silva et al. (1989).

No effects of urea supplementation on intake, *in vivo* and *in sacco* digestion and site of digestion were observed. This indicates, that N was not limiting microbial activity, although the ammonia-N concentration in the rumen of sheep fed UWS was close to the level of 50 mg/l required for maximal microbial growth *in vitro* as found by Satter and Slyter (1974) and lower than the required value of about 80 mg/l as reported by Oosting et al. (1989) for maximal *in vitro* degradation of low quality roughages. Hence, other nutrients may have been more limiting than N. The efficiency of microbial protein synthesis was low in the present experiment (Oosting et al., 1993) probably caused by limiting availability of true protein, branched chain VFA's and/or sulphur.

The contribution of the rumen to OM digestion was on average 79 %. For untreated and alkali treated straws reported values for partial rumen OM digestion vary from 54 to 80 % (Demeyer, 1981; Zorrilla-Rios et al., 1991). Supplementation with easily rumen degradable concentrates as sugar beet pulp may give higher contributions of rumen digestion to total OMD.

The contribution of digestion in the small intestine to whole tract OMD was low and ileal flows of NDF, hemicellulose and lignin seemed even higher, though not significantly, than the duodenal flows of these cell wall components. Over and within rations differences

between apparent small intestinal crude protein (N * 6.25) and non cell wall-OM disappearance were not significant. Apparent crude protein disappearance in the small intestine was 29, 34 and 53 g/day for UWS, SWS and AWS, respectively, (Oosting et al., 1993), while apparent small intestinal disappearance of non cell wall-OM was 16, 0 and 44 g/day for UWS, SWS and AWS, respectively.

The contribution of digestion in the hindgut to whole tract digestion was lower (insignificantly) for AWS than for UWS and SWS, which is confirming the suggestion by Demeyer (1991), that the importance of hindgut fermentation is increasing with decreasing quality of feeds. The partial digestion of cell wall components in the large intestine was in line with literature data summarized by Demeyer (1981). Since only 200-300 g/kg potentially degradable NDF entering the large intestine was degraded in the hindgut, the conclusion seems justified, that retention time in the hindgut was more limiting the extent of hindgut fermentation than availability of potentially degradable material.

The contribution of the large intestine to whole tract digestion was higher for OM than for NDF. This could be attributed to the fact, that the digesta entering the large intestine contained a high proportion of non cell wall OM (average over rations 36 %), with a higher apparent digestion in the large intestine (over rations 390 g/kg) than NDF (over rations 109 g/kg).

Eating and rumination behaviour

No differences were observed in eating and rumination time between rations. The rumination time was approximately 9-10 hours, about equal to the maximum daily time spent ruminating as suggested by Bosch et al. (1992) and Welch (1982). It is likely, that this maximum rumination time was required for the low quality roughages as fed in the present experiment. However, for sheep consuming low quality roughages rumination time per day was found to vary between experiments from 6 to 12 hours, though within experiments rumination time was fairly constant (Bae et al., 1979; Hogan et al., 1989; Gherardi & Black, 1989; Wales et al., 1990).

Assuming a constant efficiency of rumination (min. rumination/g NDF) and a fixed criticle particle size for escape from the rumen (Kennedy & Poppi, 1984), eating time will be restricted by maximum time for rumination. However, Gherardi & Black (1989) and Faichney (1986) suggested, that rumination efficiency may increase with higher intake of the same feed and the average size of particles leaving the rumen may also increase with increasing NDF content of the ingested feed (Bosch et al., 1992) or increasing rumen fill (Okine et al., 1990). Thus, it is unlikely, that maximum rumination time alone determines eating time and consequently intake.

The efficiency of rumination and eating (the reciprocal of time spent eating or ruminating per kg cell wall intake) was higher for AWS than for SWS and UWS. A higher efficiency of rumination and eating could mean a lower resistance to particle comminution,

but could also result in a higher average particle size in the rumen. A reduced resistance to particle size comminution due to ammonia treatment was observed by Oosting & Van Bruchem (unpublished) in cattle. Doyle (1984) reported a lower power consumption required for grinding of alkali treated rice straw than for untreated rice straw. Although particle size reduction per minute rumination time could have been more for AWS than for UWS/SWS in the present experiment, a higher proportion of rumen DM was retained on a 1.25 mm sieve in case of AWS.

No differences were found between rations in frequency of chewing during eating or rumination. More chews were recorded per minute eating than per minute chewing, which could be indicative of a higher efficiency of particle size reduction per minute chewing than per minute rumination.

The number of daily rumen contractions was not significantly different between rations and also no differences in frequency of rumen contractions during eating, rumination and resting were found. During eating the frequency of rumen contractions was higher than during rumination or resting, which could explain why passage of particles is higher during eating than during rumination (Girard, 1990), although Okine et al. (1990) observed, that duration of individual rumen contractions rather than frequency of rumen contractions determined rate of passage.

Rumen pool size

The rumen pool size is determined by the quantity of feed entering the rumen and the quantity disappearing from the rumen through passage or degradation. NDF intake was significantly higher for AWS than for the other rations, while the rumen NDF pool was not significantly different between rations. The higher rumen turnover of NDF for AWS compared with UWS/SWS was associated with an increased rumen degradation and only to a small extent with an increased passage of feed particles. Of the increased NDF intake due to ammonia treatment of 270 g/d, 91 % disappeared from the rumen due to higher rumen degradation and only 9 % due to a higher passage of NDF.

Rumen degradation and rumen and hindgut passage

In sacco studies by Adebowale et al. (1989) and Ørskov et al. (1989) showed that ammoniation of straw increased the potentially degradable fraction. The rate constant of degradation was only slightly, if at all affected in these experiments. Also in the present experiment no significant increase due to ammoniation was observed with regard to the *in sacco* rate constant of degradation of the potentially degradable fraction. The potentially degradable fraction increased, however, due to ammonia treatment, which resulted in an increased *in sacco* $k_{d,eff}$ of NDF ($k_d * f_d/(f_d + f_r)$) of 61 %. A similar increase (77 %) in $k_{d,eff}$

estimated from NDF intake, duodenal NDF flow and rumen NDF pool was found.

The estimate of k_d of NDF_d from *in sacco* analysis was over rations 2.2 %/h (for straw), while the *in vivo* estimate based on intake and duodenal flow of NDF_d was 5.2 %/h. The discrepancy between the two estimates may partly be attributed to the fact, that the *in vivo* estimate is the overall k_d of the rumen pool of NDF_d, which consisted of straw and, probably a small proportion, of sugar beet pulp with a higher k_d than for straw. Another reason for overestimation of the *in vivo* k_d is, that it was based on the assumption, that the effective passage of NDF_d was equal to the effective passage of NDF. This is only true, if the ratio NDF_d/NDF_r was equal in the large and small particle pool in the rumen and if the k_p of small particle NDF_r was equal to the k_p of small particle NDF_d. However, Tamminga et al. (1989) reported a lower k_p for NDF_d than for NDF_r and Oosting et al. (unpublished) observed a higher NDF_d/NDF_r ratio in large particles than in small particles in the rumen of cattle fed straw based rations. Both observations indicate, that the $k_{p,eff}$ of NDF_d is lower than the $k_{p,eff}$ of NDF, which would result in lower estimates of k_d.

Although the extent of overestimation of the *in vivo* k_d in the present experiment is unknown, the large deviation between the *in sacco* and *in vivo* k_d in the present experiment seems to confirm the conclusion of Aitchisson et al. (1986), that k_d derived from *in sacco* analysis underestimates *in vivo* k_d.

Although the accuracy of estimates of k_d of NDF_d in the large intestine was relatively low, the average over rations of 4.8 %/h was comparable to the estimate of 5.2 %/h for k_d in the rumen. The first order degradation model generally applied for analysis of degradation kinetics implies, that k_d is not affected by the extent to which the NDF_d was degraded. Hence, the comparable values for k_d of NDF_d in the rumen and in the large intestine indicate, that microbial degradation is of similar effectiveness in both degradation compartments.

Rumen passage of Cr-NDF and of rumen cell wall pools did not reveal significant differences between rations. Effective passage measured from actual flow of NDF was lower than when measured from Cr-NDF. Excretion curves based on Cr-NDF only describe the passage characteristics of a pool of small and indigestible particles with a high functional specific gravity. Due to entrapment of fermentation gasses in rumen particles, the functional specific gravity of rumen particles will be lower than of Cr-NDF, which may partly explain the difference in passage characteristics (Sutherland, 1987).

The effective passage of cellulose was slightly, but significantly higher than the effective passage of hemicellulose. Lignin had a higher effective passage than other cell wall components. Particles with a relatively high lignin content have probably a relatively high functional specific gravity, which may explain the relatively high effective passage. Why cellulose had a higher effective passage than hemicellulose is unknown. It could probably be associated with the fact, that leaves have a higher cellulose/hemicellulose ratio than stems (Wales et al., 1990). Leaves will probably be broken down to small particles more easily than stems.

The underestimation of $k_{l\text{-rumen}}$ by $k_{l\text{-faeces}}$ was also observed by Bosch et al. (1992). This can be attributed to the fact, that the faecal excretion pattern is the result of passage

through at least two mixing compartments *i.e.* the rumen and the hindgut. The flow rate out of a series of mixing compartments is lower than that out of one (Van 't Riet, 1988).

The retention time in the hindgut was over rations 10.0 h for Co-EDTA and 9.3 h for Cr-NDF. These observations were well in line with results from other experiments. Warner (1981) concluded, that in ruminants hardly any separation of fluid and particulate markers occurs post-abomasum. Retention time in the large intestine increased with decreasing intake level from 5 to 11 h in sheep fed lucerne chaff (Grovum & Williams, 1977). Caton et al. (1988) observed retention times in the large intestine of approximately 5 h for sheep fed a mixture of prairie hay and oat straw either with or without supplementary cottonseed meal.

Despite the higher DOMI for animals fed AWS and consequently a higher rumen VFA production, no drastic differences were observed between rations in pH and concentration and molar proportions of VFA's in the rumen. Also the rate of absorption of VFA was not significantly different between rations. The higher rumen fluid volume and the higher rate of fluid passage of animals fed AWS, although both not significantly, could probably explain the absence of differences in VFA concentration and consequently in rumen pH between the rations. In the present experiment no differences between rations were found in VFA composition. Murphy et al. (1982) related molar proportions of VFA to substrate composition, which in the present experiment, was not markedly altered by ammonia treatment. The proportion of total VFA production, that disappeared from the rumen through absorption (average 72 %) was in line with values for sheep fed roughages with an OMD of 500-600 g/kg (Ketelaars & Tolkamp, 1991) and with the estimate of 70 % for dairy cows at a maintenance level of intake (Tamminga & Van Vuuren, 1988).

It can be concluded, that the most important effects of ammonia treatment of wheat straw were an increased voluntary intake and increased degradation in the rumen. Effects on rumen pool size and rates of rumen passage and degradation were not significant. Ammonia treatment reduced the time spent ruminating per kg NDF ingested. The increased intake and digestion due to ammoniation of wheat straw did not affect the pattern of rumen fermentation end products and the relative importance of hindgut fermentation.

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3.2 Ammonia treatment of wheat straw. 2. Efficiency of microbial protein synthesis, rumen microbial protein pool size and turnover, and small intestinal protein digestion in sheep

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Abstract

Ammonia treated wheat straw (AWS) was compared with untreated wheat straw (UWS) and untreated wheat straw supplemented with urea (SWS) in an experiment with 6 wether sheep. Microbial protein synthesis increased due to ammonia treatment as an effect of the higher intake of rumen degradable organic matter. The efficiency of microbial protein synthesis was low for all rations, on average 22.1 g N/kg apparently rumen degradable organic matter and was not significantly affected by ammonia treatment. Estimates of microbial protein synthesis based on diaminopimelic acid (DAPA) or the amino acid profile method did not differ significantly. The microbial DM (average 151 g) and N pool (average 12 g) in the rumen and the proportion of rumen microbes associated with the fluid phase (average 36 %) were not affected by ration, but the clearance rate of microbes tended ($p < 0.10$) to be higher for AWS (2.9 %/h) than for UWS (2.1 %/h) and SWS (2.2 %/h). True rumen degradation of dietary amino acid-nitrogen (AA-N) was not affected by ration (average 412 g/kg). Small intestinal apparent digestibilities of N, AA-N, amino acids and non-protein nitrogen (NPN) were not significantly different between rations and were on average 485, 583, 593 and 313 g/kg, respectively. Apparent small intestinal AA-N absorption was significantly higher for AWS (6.0 g/d) than for UWS (3.8 g/d) and SWS (4.2 g/d).

Keywords: efficiency of microbial protein synthesis, rumen microbial pool, small intestinal protein availability.

Introduction

In ruminants, the supply of amino acids for maintenance and production depends on the availability of microbial protein and undegraded dietary protein for digestion in the small intestine. The quantity of crude protein ingested as such is only of minor importance in that respect. Microbial protein synthesis is closely related to the quantity of fermentable organic matter ingested and the amount and nature of the N source available. In low-quality fibrous feeds, crude protein content is low. About half is degraded in the reticulo-rumen and the small intestinal digestibility of the part escaping from reticulo-rumen degradation is presumed to be limited (Hvelplund, 1989), even after ammonia treatment. Hence, with such low quality fibrous diets, microbial protein synthesized in the reticulo-rumen constitutes almost the only source for protein digestion in the small intestine.

The efficiency (microbial N yield/kg apparently rumen degraded OM) of microbial protein synthesis in the rumen is related to nutrient availability and retention time of microbes in the rumen (Hespell & Bryant, 1979). Inadequacy of nutrients as sulphur, phosphorus, true protein and branch chained volatile fatty acids can limit microbial growth without affecting energy fermentation. In addition, a longer retention time of microbes in the rumen results in a higher turnover of microbial matter due to increased lysis and predation by protozoa.

Of the protein entering the duodenum, an average true digestibility has been reported of about 85 % (Van Bruchem et al., 1989). The actual supply of amino acids, however, is more closely related to the amino acid's apparent digestibility. The difference between these two digestibility measures is caused by the quantity of endogenous protein lost from the small intestine, which in turn is related to the extent at which endogenous protein is produced in the small intestine as enzymic protein, mucoprotein and shredded epithelial cells. Apparently, a considerable part of this endogenous protein is re-utilized in the small intestine (Van Bruchem et al., 1987), thus salvaging part of the constituent amino acids.

It was previously suggested that the net endogenous protein production is positively related to the quantity of cell wall constituents passing the small intestine (Van Bruchem et al., 1989). This could be of particular relevance with the highly fibrous diets fed in the present experiment. An increased endogenous protein production on top of a relatively low quantity of protein reaching the duodenum could result in an extremely low apparent protein digestibility in the small intestine. In such a situation the quantity of amino acids coming available for intermediary metabolism would particularly become a constraint, even for moderate levels of production, such as for example early growth (Ørskov, 1982)

The present paper describes the efficiency of microbial protein synthesis and the apparent small intestinal digestion of amino acids in sheep fed wheat straw as the basal diet, either supplemented with urea or treated with ammonia.

Materials and methods

The major part of materials and methods of the present experiment was described in detail by Van Bruchem et al. (1993). A summary is given below. Materials and methods not described by Van Bruchem et al. (1993) are given in detail.

Six mature sheep (wethers) with an average live weight of 44 kg were fitted with a rumen cannula, a silastic infusion tube in the abomasum and with T-shape hard pvc cannulas in the proximal duodenum and terminal ileum. The experiment started 2.5 months after surgery, after the animals had well recovered.

During the experiment the animals were kept in metabolic cages and received equal portions of their ration every four hours. Water and a mineral lick containing NaCl, Fe, Mg and Co were freely available.

The animals were randomly allotted into three groups of two sheep each. Group one was fed untreated wheat straw *ad libitum* supplemented with pelleted sugar beet pulp (UWS). Group two was fed the same ration with an infusate into the rumen of an urea solution (60 ml/h; concentration of 5.4 g urea-N/l; 7.9 g N daily) (SWS) and group three was fed ammoniated wheat straw *ad libitum* also with a supplement of pelleted sugar beet pulp (AWS).

The untreated and ammoniated wheat straws were offered in a quantity of 1.8 kg daily allowing selection. The sugar beet pulp was supplied at a level of 240 g product daily. The composition of the feeds with regard to N and amino acid nitrogen (AA-N) is given in Table 1.

Table 1. N and AA-N concentration (g/kg) in untreated wheat straw, ammoniated wheat straw and sugar beet pulp.

	Untreated wheat straw	Ammoniated wheat straw	Sugar beet pulp
DM	887	843	886
N[1]	6	18	13
AA-N[1]	4	4	11
AA-N/N (g/kg)	619	207	829

[1] In DM

Before the start of the experiment the animals were fed untreated wheat straw during 5 weeks. The experiment consisted of a two week adaptation period and a seven week experimental period. The results presented here were derived from measurements during weeks 3, 4 and 7 of the experimental period.

In the 3rd and 4th week of the experiment the flow of nutrients in the duodenum and ileum was assessed based on a continuous infusion of about 20 mg Co and 20 mg Cr per hour into the abomasum, starting one day prior to sampling. The composition of the infusate and the sampling procedure were as described by Van Bruchem et al. (1993). Hourly samples of approximately 10 g of duodenal and ileal digesta were collected during four days. After collection, the samples were freeze dried, ground and pooled per animal, week and cannula. In addition to the constituents already described by Van Bruchem et al. (1993), samples were analyzed for N and amino acids including diaminopimelic acid (DAPA). Duodenal and ileal flows were estimated based on the concentration of a constituent relative to the concentration of markers. N was determined by the Kjeldahl method with K_2SO_4 and $CuSO_4$ as catalysts. Amino acids, including DAPA were determined as described by Van Bruchem et al. (1988) with a Biotronic LC5001 automatic amino acid analyzer. Samples were hydrolysed under reflux with HCl (6 mol/l) at 110°C for 22 h. The sulphur containing amino acids methionine and cystine were determined as methionine sulphone and cysteic acid, respectively, after performic acid oxidation (Moore, 1963).

During the morning of each sampling day, a composite sample of rumen fluid was collected from the ventral rumen sac. From these samples, rumen microbes were isolated by differential centrifugation (550-70,000 g) with an MSE superspeed 65 centrifuge at 4°C. The pellet was washed twice with a buffer, prepared according to Meyer et al. (1967). After freeze-drying, the individual amino acids including DAPA and N were determined, the latter with the micro-Kjeldahl method.

Overall digestibility of N was measured by daily collection and subsampling of residual feed and total collection of faeces over six days in weeks 3 and 4. Faecal N excretion was not corrected for duodenal and ileal sampling, which was done during four of the six days of faecal collection. The N withdrawal from the duodenal and ileum was approximately 250 mg/d, which, averaged over the faecal collection period means an underestimation of faecal N excretion of maximally 170 mg/d.

During the 7th week of the experiment total rumen evacuations were conducted in such a schedule, that of all animals total rumen contents were measured and a proportional sample for analyses of DM and DAPA was taken at 1, 2 and 3 hours after feeding. Analyses were done in samples pooled per animal. A sample of the fluid phase was taken by straining whole rumen contents over cheese cloth. After pooling per animal, these samples were analyzed for DM and DAPA. The DAPA pool associated with rumen fluid was calculated as concentration of DAPA in rumen fluid times rumen fluid pool (rumen contents minus rumen DM pool). The DAPA pool in the particulate phase was estimated by subtracting the fluid phase DAPA pool from the total rumen DAPA pool. Microbial N and DM pools were

estimated by multiplication of the DAPA pool with the N/DAPA- or DM/DAPA-ratio as found in isolated rumen microbes.

Statistical analysis was done on means per animal (average of two repeated measurements) by the program DBSTAT (Brouwer, 1989). The model to test the ration effect on observed or estimated parameters (Y) was: Y_{ij} = mean + $ration_i$ + $error_{ij}$, with total degrees of freedom (d.f.) 6. To test the significance of contrasts: L1 : -1*UWS - 1*SWS + 2*AWS and L2 : -1*UWS + 1*SWS the ration sum of square was subdivided in the sum of square of each contrast with d.f. = 1. The d.f. of the error term was 3. (Snedecor & Cochran, 1967). The level of significance of a contrast was indicated in tables for $p < 0.25$ (§), $p < 0.10$ (†), $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

Results

Rumen microbes

Table 2 presents the composition of rumen microbes. The microbes in the rumen of sheep fed AWS tended ($p < 0.10$) to contain less N, AA-N and total amino acids in the DM, while the DAPA concentration in microbial DM of sheep fed AWS was significantly lower than of sheep fed UWS or SWS. The ratio AA-N/total N tended ($p < 0.10$) to be lower in microbes of sheep fed AWS than of microbes of sheep fed UWS or SWS. The ratio DAPA-N/total microbial N was 6.1, 6.8 and 5.5 g/kg (SEM 0.32) for UWS, SWS and AWS, respectively. Ammonia treatment tended ($p < 0.10$) to decrease the DAPA-N/total N ratio in rumen microbes.

In Table 3, the microbial DM and N pool in the rumen is given as well as the distribution of microbial matter between fluid and particulate phases. The rate of clearance of microbial matter from the rumen ($k_{cl,microbes}$) was calculated as the hourly DAPA flow in the duodenum as percentage of the rumen DAPA pool.

No significant differences between rations were observed for microbial DM or N pool. The microbial DM pool contributed for 179, 151 and 174 g/kg to the total rumen DM pool for UWS, SWS and AWS, respectively ($p > 0.1$). The proportion of total microbial DAPA, that was associated with the fluid phase was not different between rations and averaged 36.0 %. The concentration of microbial N in rumen fluid was not significantly different between rations. The rate of clearance of rumen microbes from the rumen microbial pool tended ($p < 0.10$) to be higher for AWS than for UWS and SWS.

Table 2. Composition of rumen microbes

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
DAPA (mmol/kg DM)	18	20.5	14.9	*	§	0.94
Amino Acids (mol/kg DM)	3.5	3.6	3.1	†	ns	0.14
N (g/kg DM)	83	84	76	†	ns	2.4
AA-N (g/kg DM)	60	62	54	†	ns	2.3
AA-N/N (g/kg)	729	737	715	†	ns	5.4

ns: not significant, §: $p < 0.25$, †: $p < 0.10$, *: $p < 0.05$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

Table 3. Size, proportion associated with liquid phase and rate of clearance of rumen pool of microbes.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
Microbial DM pool (g)	136	137	179	§	ns	18.1
Microbial N pool (g)	11.2	11.5	13.6	§	ns	1.27
Microbial pool associated with fluid (% of rumen microbial pool)	35.9	35.2	37.0	ns	ns	1.60
Concentration of microbial N in rumen fluid (mg/l)	735	636	597	ns	ns	84.9
$k_{cl,microbes}$ (%/h)	2.1	2.2	2.9	†	ns	0.22

ns: not significant, §: $p < 0.25$, †: $p < 0.10$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.

The quantity of microbial protein synthesized in the rumen was determined by different methods:

1) DAPA: The DAPA flow (mole/day) in the duodenum was multiplied with the N/DAPA-ratio (g/mole) as found in isolated rumen microbes.
2) Amino Acid Profile method (AAP): Dietary, microbial and endogenous proteins

(Table 4) were mixed by an iterative procedure in such proportions, that the computed AAP matched best to the actual AAP of duodenal protein. This was tested by minimizing the objective function:

$$\sum_{AA=1}^{AA=16} (1-AA_{computed}/AA_{actual})^2$$

This procedure was done for 16 amino acids (AA), leaving out cystine, due to the high analytical variation. The AAP is given in molar percentages and the AAP of endogenous protein (bovine pepsinogen) was adapted from Siddons et al. (1982). Since only marginal and insignificant differences were observed for the AAP of diets and rumen microbes between animals and rations the calculation of proportions of feed, microbial and endogenous AA-N in duodenal digesta was done on the average of all diets and rumen microbes. The obtained partition of duodenal AA-N in endogenous, microbial and feed AA-N is given in Table 4.

The efficiency of microbial protein synthesis is presented in Table 5. No significant differences were observed between rations. No differences between methods of estimation (DAPA or AAP) of the efficiency of microbial protein synthesis were found. For the results based on DAPA the Y_{ATP} (g cell DM/mol ATP) was calculated from the efficiency of microbial protein synthesis per kg TRDOM (truly rumen degradable organic matter) ingested (Van Soest, 1982). Per kg TRDOM 28.4 moles of ATP are produced, assuming a molecular weight of glucose in carbohydrates of 162 and an ATP formation of 4.6 moles/mol glucose (Czerkawski, 1986). Calculation of the latter value was based on the volatile fatty acid pattern found in rumen fluid (Van Bruchem et al., 1993).

The maintenance coefficient of the microbial population (M_e, mol ATP/g cell DM/h) was estimated from the model:

$$1/Y_{ATP} = 1/Y_{ATP}^{MAX} + M_e/k_{cl,microbes},$$

with:

Y_{ATP}^{MAX}: maximal cell DM yield (g) per mol ATP

$k_{cl,microbes}$: rate of clearance of microbes from the rumen, expressed as fraction/h. (adapted from De Vries et al., 1970).

Table 4. Amino acid profiles (molar %) of endogenous, dietary, rumen microbial and duodenal (D-UWS, D-SWS and D-AWS) proteins and proportion of amino acids of microbial, feed and endogenous origin in duodenal digesta.

	Endo-genous[1]	Diet[2]	Microbes[2]	D-UWS	D-SWS	D-AWS
Cystine	n.a.	1.26	0.70	1.22	1.10	1.12
Aspartic acid	11.50	9.48	11.37	10.58	10.65	11.11
Methionine	1.08	2.00	2.12	1.63	1.70	1.70
Threonine	7.63	5.90	6.40	6.36	6.41	6.41
Serine	14.32	7.35	5.90	6.68	6.55	6.55
Glutamic acid	9.22	10.77	10.49	10.27	10.39	10.20
Proline	4.43	4.84	3.67	4.66	4.24	3.78
Glycine	9.94	9.52	9.23	9.35	9.40	9.44
Alanine	4.60	9.01	10.34	9.87	10.10	9.75
Valine	7.12	7.01	6.93	6.62	6.97	6.77
Isoleucine	9.01	4.19	5.75	5.28	5.35	5.39
Leucine	7.20	6.63	7.07	7.33	7.33	7.18
Tyrosine	5.08	3.40	3.60	3.35	3.27	3.31
Phenylalanine	4.29	3.36	3.80	3.72	3.75	3.68
Lysine	2.30	4.85	6.55	6.21	5.49	6.34
Histidine	0.56	6.15	2.66	3.48	3.78	3.95
Arginine	1.72	4.28	3.42	3.38	3.50	3.34
Proportion of duodenal AA:						
microbial AA				62	57	64
feed AA				30	36	31
endogenous AA				8	7	5

1 Composition of bovine pepsinogen adapted from Siddons et al. (1982).
2 Mean for the 3 rations.
n.a. not available.

The above given equation implies, that *in vivo*, given a constant Y_{ATP}^{MAX}, the net Y_{ATP} is determined by $k_{cl,microbes}$ and M_e. The $k_{cl,microbes}$ determines the retention time of microbes in the rumen and consequently the probability of predation by protozoa, which is the major determinant of microbial turnover in the rumen (Demeyer & Van Nevel, 1979). To get an estimate for Y_{ATP}^{MAX} the model was fitted to *in vitro* data derived from continuous cultures of mixed rumen microbes at various rates of clearances with glucose as the sole energy substrate as reported by Isaacson et al. (1975) and Van Nevel & Demeyer (1979). The regression line obtained was: $1/Y_{ATP} = 0.0508 + 0.00126/k_{cl,microbes}$ (n=7, $R^2 = 0.88$, RSD = 0.007), indicating an Y_{ATP}^{MAX} of 19.7 g cell DM/mol ATP, close to the theoretically derived estimate of 22-24 g cell DM/mol ATP given by Hespell & Bryant (1979) and a maintenance coefficient of 1.26 mmol ATP/g cell DM/h.

Based on the Y_{ATP}^{MAX} estimated for rumen microbes in continuous cultures and the $k_{cl,microbes}$, the M_e of the microbial populations was calculated for the various rations.

Table 5. Efficiency of microbial protein synthesis and rumen degradability of feed AA-N.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
Efficiency microbial protein synthesis (g N/kg ARDOMI[1]):						
- *DAPA*	24.1	22.8	19.4	§	ns	2.15
- *AAP*	25.0	22.0	18.5	§	ns	2.45
Y_{ATP} (g cell DM/mol ATP)[2]	8.0	7.7	7.4	ns	ns	0.45
M_e[3] (mmol ATP/g cell DM/h)	1.6	1.7	2.5	*	ns	0.13
Rumen degradability of feed AA-N (g/kg)						
- *DAPA*	422	444	463	ns	ns	59.9
- *AAP*	463	368	406	ns	§	36.2

ns: not significant, §: $p < 0.25$, *: $p < 0.05$.
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.
1 ARDOMI: Apparently rumen degraded organic matter intake.
2 Based on DAPA.
3 Based on DAPA. M_e = Maintenance coefficient of microbial population.

The M_e was significantly higher for AWS than for UWS and SWS.

No difference between rations was observed with regard to the rumen degradation of feed AA-N. The AA-N degradation was computed assuming an endogenous AA-N flow in the duodenum as derived from the AAP method. The microbial AA-N flow was calculated from the AAP method or the DAPA method and the duodenal feed AA-N flow was equal to total AA-N flow minus endogenous and microbial AA-N flow.

Nitrogen supply

Intake, duodenal and ileal flows and faecal excretion of OM, N and AA-N are presented in Table 6. Intake of OM, N and AA-N of AWS was significantly higher than of UWS and SWS. The N intake from SWS was 8.4 g/day higher than from UWS of which 7.9 g/day was due to infusion of urea into the rumen. Duodenal flow of OM tended ($p < 0.10$) to be higher for AWS than for UWS and SWS and duodenal flows of N and AA-N were significantly higher for AWS than for UWS and SWS. The duodenal flows of N and AA-N in case of UWS and of AA-N in case of SWS and AWS were higher than the intake of these nutrients, indicating the importance of influx of N into the rumen through saliva and diffusion through the rumen wall in case of UWS and the incorporation of ammonia into amino acids by rumen microbes for all rations.

Table 6. Intake and flow at duodenum and ileum and faecal excretion of OM, N and AA-N.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
Intake (g/day)						
OM	550	602	940	*	ns	58.4
N	4.6	13.0	19.9	***	**	0.70
AA-N	3.7	4.1	5.3	*	ns	0.24
Duodenal flow (g/day)						
OM	316	340	447	†	ns	41.5
N	9.7	11.0	17.5	**	ns	0.92
AA-N	6.7	7.2	10.1	*	ns	0.63
microbial[1]	*4.2*	*4.1*	*6.5*	*	ns	0.37
feed[1]	*2.0*	*2.6*	*3.1*	†	§	0.21
endogenous[1]	*0.5*	*0.5*	*0.5*	ns	ns	0.04
AA-N/N	0.69	0.65	0.58	*	§	0.018
Ileal flow (g/day)						
OM	311	346	410	§	ns	40.7
N	5.0	5.6	9.1	*	ns	0.55
AA-N	2.9	3.0	4.1	†	ns	0.34
AA-N/N	0.59	0.52	0.45	*	†	0.019
Faecal excretion (g/day)						
OM	242	274	335	§	ns	36.7
N	3.6	3.9	7.2	**	ns	0.31

ns: not significant, §: $p < 0.25$, †: $p < 0.10$, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.
1 Based on AAP

The microbial, feed and endogenous AA-N flows in the duodenum as estimated by the AAP method are also given in Table 6. The microbial AA-N flows estimated by DAPA were: 4.0, 4.4 and 6.8 g/day for UWS, SWS and AWS respectively (SEM 0.31). The microbial AA-N ($p < 0.05$) and the undegraded feed AA-N ($p < 0.10$) flows in the duodenum were higher for AWS than for UWS and SWS. No significant differences in endogenous AA-N flows were found between rations. The ratio AA-N/N in the duodenum was significantly lower in AWS than in UWS and SWS.

The ileal flow of N was significantly higher for AWS than for UWS and SWS, while the ileal flow of AA-N tended ($p < 0.10$) to be higher for AWS than for UWS and SWS. The AA-N/N ratio in the ileum differed significantly between rations and was highest for UWS and lowest for AWS.

The apparent absorption of AA-N in the small intestine was 3.8, 4.2 and 6.0 g/day (SEM 0.30) for UWS, SWS and AWS, respectively. The apparent daily absorption was significantly higher for AWS than for UWS and SWS.

The faecal excretion of N in case of AWS was significantly higher than in case of UWS and SWS. Some disappearance of N occurred in the large intestine: 1.4, 1.7 and 1.9 g/day or 286, 298 and 203 g/kg ileal N flow for UWS, SWS and AWS, respectively. These values did not differ significantly between rations. The disappearance of N in the hindgut was for all rations more than the maximal underestimation (due to withdrawal of N through duodenal and ileal sampling) of faecal N excretion of maximally 170 mg/day.

Whole tract apparent N digestion was 228, 697 and 635 g/kg N ingested for UWS, SWS and AWS, respectively. UWS had a significant lower apparent whole tract N digestibility than SWS and AWS, while SWS and AWS did not differ significantly. True whole tract N digestibility was estimated from the following Lucas equation (Van Soest, 1982):

$DN = a*N - b$

with DN = intake of digestible N (% of OMI), N = intake of N (% of OMI), b = metabolic faecal N (% of OMI) and a = true digestibility of N (fraction).

The model was fitted to all data including the repeated measurements. The variation in N concentration in OMI was mainly caused by N added through ammonia treatment or urea supplementation. Hence, fitting of the model for UWS and SWS gave an estimate for the true digestibility of N added through the urea infusate and fitting of the model for UWS and AWS gave an estimate for the true digestibility of N added through ammonia treatment. The obtained Lucas equations were (between brackets s.e. of estimate): DN = -0.65 (0.029) + 1.00 (0.017) * N for N added through the urea infusate and DN = -0.56 (0.045) + 0.89 (0.028) * N for N added through ammonia treatment. The true digestibility of N added through ammonia treatment was significantly lower than 1.

The apparent small intestinal digestibility of N, AA-N, NPN, individual and total amino acids is given in Table 7. No differences were observed between rations, only phenylalanine had a significantly lower apparent small intestinal digestibility for UWS than for SWS and AWS. The apparent small intestinal digestibility of AA-N was higher than of NPN.

Van Bruchem et al. (1989) related ileal endogenous protein losses to the duodenal non protein dry matter (NPDM) flow. The duodenal NPDM flows in the present experiment were 371, 393 and 518 g/d for UWS, SWS and AWS, respectively (SEM 30.1). Assuming a true small intestinal digestibility of AA-N of 0.85 as observed by Van Bruchem et al. (1989), Hvelplund & Hesselholt (1987) and Storm et al. (1983) the endogenous AA-N flows (ileal flows - 0.15*duodenal flows) at the ileum were 5.1, 4.9 and 5.0 mg/g NPDM for UWS, SWS and AWS, respectively (SEM 0.08).

Table 7. Apparent digestibility (g/kg) of AA-N, N, NPN and individual amino acids in the small intestine.

	UWS	SWS	AWS	Significance of contrast		SEM
				L1	L2	
N	490	490	476	ns	ns	17.0
AA-N	566	589	595	ns	ns	14.2
NPN	324	303	313	ns	ns	29.6
Cystine	171	127	246	ns	ns	56.1
Aspartic acid	566	606	630	§	§	19.8
Methionine	700	686	705	ns	ns	34.4
Threonine	532	549	573	§	ns	16.6
Serine	492	516	509	ns	ns	24.9
Glutamic acid	568	596	607	ns	ns	15.3
Proline	578	570	505	ns	ns	54.7
Glycine	510	537	546	ns	ns	21.0
Alanine	562	569	592	§	ns	14.1
Valine	608	638	646	§	§	13.1
Isoleucine	665	695	701	ns	ns	19.3
Leucine	629	664	669	ns	ns	18.7
Tyrosine	596	638	649	†	†	10.9
Phenylalanine	620	668	675	*	*	8.0
Lysine	674	646	710	ns	ns	17.0
Histidine	280	415	335	ns	§	49.8
Arginine	610	639	662	ns	ns	22.8
TAA[1]	572	598	608	§	§	11.2

ns: not significant, §: $p < 0.25$, †: $p < 0.10$, *: $p < 0.05$
L1: contrast -1*UWS - 1*SWS + 2*AWS.
L2: contrast -1*UWS + 1*SWS.
1 Total amino acids

Discussion

Rumen microbes

Rumen microbes isolated from the rumen of sheep fed AWS contained significantly less DAPA and tended ($p < 0.10$) to contain less amino acids, N and AA-N in DM. The ratio DAPA-N/total microbial N was also lower ($p < 0.10$) for AWS than for UWS and SWS. The DAPA-N/total microbial N ratios as observed in the present experiment were in line with results published by Dufva et al. (1982), Siddons et al. (1982) and Van Bruchem et al. (1985). The composition of rumen microbes is influenced by the ration as illustrated by Dufva et al. (1982), who observed that the N concentration in microbial DM and the DAPA-N/total microbial N was higher for rumen microbes isolated from the rumen of cattle

fed a high roughage ration than for rumen microbes isolated from the rumen of cattle fed a high concentrate ration. Variation in DAPA-N content of total microbial N can probably be attributed to variation in relative contribution of various microbial strains to the microbial pool, since microbial strains may differ in DAPA-N/total microbial N ratio (Dufva et al., 1982). The lower N, AA-N and amino acid content in DM of rumen microbes with AWS as substrate could probably also be an indication of a different microbial population, but could also be attributed to a higher content of polysaccharides in individual microbial strains as this is likely to vary with ration (Czerkawski, 1986). The higher maintenance requirements for the microbial population in the rumen of sheep fed AWS compared with UWS/SWS could also be a result of the different composition of the rumen microbial pool. Microbial strains differ with regard to maintenance requirements (Hespell & Bryant, 1979). However, individual strains of rumen microbes may show increased maintenance requirements in situations of imbalanced nutrient availability *i.e.* limited supply of N, sulphur, phosphorus or true protein relative to energy availability (Nocek & Russell, 1988). Whether the higher maintenance requirements of the rumen microbial population for AWS compared with UWS/SWS should be attributed to a different composition of the rumen microbial pool or to imbalanced nutrient supply is unknown.

Though the composition of rumen microbes differed, the amino acid profiles of rumen microbes did not differ between rations, and were neither differing much from microbial amino acid profiles found in other experiments (Siddons et al., 1982; Van Bruchem et al. (1985 and 1988), Storm & Ørskov, 1983; Hvelplund & Hesselholt, 1987; Tas et al., 1981).

The microbial biomass associated with rumen fluid phase was on average 36.0 % of the total microbial biomass. In a review of various experiments, Owens & Goetsch (1986) reported, that the proportion of the microbial biomass associated with the fluid phase varied from 20 to 47 %. The remaining fraction of the microbial biomass is associated with feed particles and the rumen wall (Cheng & Costerton, 1980).

Ammonia treatment tended to increase the clearance rate of rumen microbes. Ammonia treatment affected neither the rate of clearance of particles from the rumen (Van Bruchem et al., 1993) nor the distribution of the microbial pool between the fluid and particulate phase. The higher clearance rate of rumen microbes in case of AWS should therefore partly be attributed to passage rate of fluid, which was 20 %, though not significantly higher (Van Bruchem et al., 1993). However, differences between rations in concentration of microbes on particles passing from the rumen could also result in different clearance rates of rumen microbes.

The concentration of microbial N (on average 656 mg/l) was much higher than the concentration of NH_3-N in rumen fluid, which was 43, 225 and 175 mg/l for UWS, SWS and AWS, respectively (Van Bruchem et al., 1993)

Efficiency of microbial protein synthesis

Calculation of the proportion of feed, endogenous and microbial amino acids in the

duodenal digesta with the amino acid profile method yielded similar results for all rations. The average proportions of microbial, endogenous and feed amino acids in the total amino acid flow in the duodenum were respectively 61, 32 and 7 %. The amino acid profile method is based on many assumptions, which may not always be valid as discussed by Siddons et al. (1982). In addition, the endogenous fraction is likely to contain proteins from other sources than pepsinogen (Harrop, 1974) and feed protein arriving at the duodenum may have a different amino acid profile than the feed ingested.

Estimation of the microbial AA-N flow at the duodenum by DAPA or the AAP method were not differing significantly as also observed by Evans et al. (1975) and Van Bruchem et al. (1985). However, Siddons et al. (1982) and Voigt et al. (1991) observed lower estimates of microbial AA-N production for the amino acid profile method than for DAPA, while van Bruchem et al. (1988) observed higher values for the amino acid profile method than for DAPA.

The observed efficiencies of microbial N synthesis (averaged over rations 22.1 and 21.8 g N/kg ARDOM for DAPA and AAP, respectively) were low if compared with the constant of 32 g N/kg ARDOM as given by ARC (1980) for sheep and with values observed in sheep by Van Bruchem et al. (1985) for diets including mixed concentrates with proteins varying in rumen degradability. However, the efficiency of microbial N in the present experiment did not differ from estimates based on DAPA reported by Van Bruchem et al. (1988) for alfalfa and grass silages fed to sheep and estimates given by Zorrilla-Rios et al. (1991) for untreated wheat straw fed to cattle. The latter authors observed, however, a higher efficiency for ammoniated wheat straw than for untreated wheat straw, which was not observed in the present experiment.

Urea supplementation did not increase the efficiency of microbial protein synthesis, indicating, that NPN was not the first limiting nutrient for microbial protein synthesis. Other nutrients essential for microbial protein synthesis as sulphur and phosphorus (not supplemented in the present experiment) and true protein as a source of oligo-peptides and branch chained volatile fatty acids (Hespell & Bryant, 1979) could have been more limiting. From the true rumen degradation of feed AA-N it was calculated, that 59, 63 and 67 % (SEM 4.2) of the microbial AA-N flow in the duodenum was produced from NPN for UWS, SWS and AWS, respectively. Nolan & Stachiw (1979) observed, that 62 % of the microbial N was derived from ammonia for sheep on a wheat straw based diet. These values suggest, that 30-40 % of microbial protein should be synthesized from true protein. It was therefore likely, that rumen availability of true protein was limiting microbial protein synthesis in the rumen.

Efficiency of microbial protein synthesis increases with increasing flow rate of microbes due to a reduction in microbial turnover (due to predation by protozoa) and decreases with increasing maintenance requirements of the microbial population (De Vries et al., 1970; Hespell & Bryant, 1979). The flow rate of microbes is related to the flow rate of fluid and particulate phase (Oldham, 1984). Although on average 64 % of the microbial pool was associated with the particulate phase in the rumen, the contribution of fluid phase

associated microbial matter to the duodenal flow of microbes may be equally or more important than the contribution of the particulate phase associated microbes. The flow rate of the fluid phase associated microbes is probably closely related to the fractional flow rate of the fluid phase, which was higher than the fractional flow rate of the particulate phase (Van Bruchem et al., 1993). In addition, of the cell walls passing from the rumen approximately 75 % of the potentially degradable fraction was degraded in the rumen (Van Bruchem et al., 1993). It is therefore likely, that the concentration of microbial matter on particles leaving the rumen was lower than on particles in the rumen.

Digestibility of AA-N and N.

The true rumen digestibility of feed AA-N was on average 412 g/kg AA-N ingested (based on AAP method) without significant differences between rations. The AA-N/N ratio in duodenal digesta was lower for AWS than for UWS and SWS, indicating, a relatively higher NPN flow for AWS than for the other rations. Duodenal NPN flows were 3.0, 3.8 and 7.4 g/d (SEM 1.0) for UWS, SWS and AWS, respectively. Ammonia-N flows from the rumen were 0.4, 1.9 and 2.7 g/d for UWS, SWS and AWS, respectively (Van Bruchem et al., 1993) and could only partly explain the difference in NPN flow between rations. Other factors, that could contribute to the higher NPN flow found with AWS compared with UWS/SWS were the lower AA-N/N ratio found in rumen microbes for AWS and the fact, that part of the N added through ammonia treatment was not available. The latter was confirmed by the fact, that the true whole tract digestibility of N added through ammonia treatment was significantly lower than 1.

The apparent small intestinal digestion of amino acids and AA-N did not differ between rations. Van Bruchem et al. (1989) reported the following relation between duodenal amino acid and duodenal NPDM flow and ileal AA-N flow based on a number of experiments in which various roughages and roughage/concentrate mixtures were fed to sheep:

Ileal amino acid flow (mmol/h) = 2.498 + 0.152 * duodenal amino acid flow (mmol/h) + 0.4134 * duodenal NPDM flow (g/h).

This formula indicates, that the true small intestinal digestibility of amino acids is 848 mmol/mol and that the endogenous amino acid flow in the ileum is related to the NPDM flow in the duodenum. The apparent small intestinal amino acid digestibility is thus negatively related to the NPDM and positively related to the amino acid flow in the duodenum. A positive correlation between amino acid flow in the duodenum and the apparent small intestinal digestibility of amino acids was also found by Willms et al. (1991).

With a relatively low duodenal amino acid flow and a high NPDM flow a low apparent small intestinal amino acid digestibility in the small intestine is expected. Based on the equation given above the apparent small intestinal amino acid digestibilities in the present

experiment would be 30, 31 and 38 % for UWS, SWS and AWS, respectively. The observed apparent small intestinal amino acid digestibilities were 57, 60 and 61 % for UWS, SWS and AWS, respectively. A lower ileal flow of endogenous amino acids could account for the difference between the estimates based on the equation of Van Bruchem et al. (1989) and the observed values in the present experiment. Recalculation of the results of Van Bruchem et al. (1989) reveals an ileal endogenous AA-N flow of 7.9 mg/g NPDM as derived from a model without intercept. Assuming a true digestibility of duodenal AA-N of 85 %, then ileal endogenous AA-N losses would amount on average 5.0 mg/g NPDM. This value would become even lower if the true digestibility of undegraded straw protein would be lower than 85 % as suggested by Hvelplund (1989). It thus seems, that sheep fed on these low quality feeds adapt their amino acid requirements by lowering ileal losses of endogenous protein.

The value for the apparent small intestinal AA-N digestibility of SWS corresponds with the apparent small intestinal digestibility of non-ammonia-N (NAN) of 58 % reported by Zorrilla-Rios et al. (1991) for untreated wheat straw supplemented with urea fed to cattle. These authors reported, however, a lower small intestinal degradability of NAN for ammoniated wheat straw of 48 % for ammoniated wheat straw than for urea supplemented wheat straw. Willms et al. (1991) observed an apparent small intestinal digestion of amino acids for alkaline hydrogen peroxide treated wheat straw supplemented with soybean meal fed to sheep of 45.7 % for the lowest supplementation level (8.5 % CP in the ration) used in their experiment. In this situation the duodenal NPDM and amino acid flow approximated the values for AWS. Cecava et al. (1990) observed slightly higher apparent small intestinal amino acid digestibilities, ranging from 60.6 to 67.2 % for alkaline hydrogen peroxide treated wheat straw supplemented with various protein sources fed to sheep. The duodenal amino acid flows in this experiment were, however, much higher than for AWS in the present experiment.

The quantity of AA-N apparently absorbed from the small intestine was significantly higher for AWS than for UWS and SWS. Since the apparent digestibility of AA-N in the small intestine did not differ between rations, the apparent AA-N absorption was largely determined by the duodenal flow of AA-N. In turn, the difference in duodenal flow between rations was mainly determined by a difference in microbial AA-N flow in the duodenum, and, since the efficiency of microbial protein synthesis did not differ between rations to the amount of OM apparently fermented in the rumen.

The endogenous AA-N flow in the proximal duodenum did not differ between rations. Zorrilla-Rios et al. (1991) also did not observe differences between endogenous N flow in the duodenum between ammoniated wheat straw and wheat straw supplemented with urea.

The small intestinal apparent digestibility of individual amino acids did not differ between rations, except for phenylalanine, which was lower for UWS than for AWS and SWS. The apparent digestibilities of cystine and histidine were low. Storm et al. (1983) observed a low true digestibility of microbial cystine and histidine.

Absorbed amino acids will enter the amino acid pool and can be utilized for tissue

maintenance and production. Formation of endogenous protein and supply of N for microbial protein synthesis can probably be covered by the N lost in tissue maintenance processes as postulated by Ørskov (1982). Thus the total of urinary and faecal N excretion of animals fed on N free diets should be regarded as net maintenance requirements. A value of 250 - 400 mg AA-N/$kg^{0.75}$/d was proposed by Ørskov (1982). Accepting an efficiency of utilization of truly absorbed AA-N of 0.65 (Rohr & Lebzien, 1991) the maintenance requirements for truly absorbed AA-N are therefore in the range of 380 - 620 mg/$kg^{0.75}$/d. In an experiment with sheep fed untreated and ammoniated wheat straw, the latter either unsupplemented or supplemented with casein or potato protein, Van Bruchem et al. (unpublished) obtained by regression of truly absorbed AA-N on N balance as estimate of maintenance requirements for AA-N 520 mg/$kg^{0.75}$/d. In the present experiment, based on a true small intestinal digestibility of AA-N of 0.85 estimates of truly absorbed AA-N were 337, 360 and 490 mg AA-N/$kg^{0.75}$/d (SEM 19.2) for UWS, SWS and AWS, respectively. This suggests, that maintenance requirements for AA-N were not met for UWS and SWS and could approximately be covered for AWS. As reported by Van Bruchem et al. (1993) the digestible organic matter intake of the sheep in the present experiment was 70, 75 and 133 % of the maintenance requirements for UWS, SWS and AWS, respectively. Comparison of estimates of energy and AA-N availability from the rations in the present experiment indicate, that the AA-N/energy ratio was more or less balanced in case of UWS and SWS, but that for AWS availability of truly absorbed AA-N could only sustain maintenance, while energy intake was above maintenance.

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3.3 Intake and utilization of energy from ammonia-treated and untreated wheat straw by steers and wether sheep fed a basal diet of grass pellets and hay

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Abstract

Two experiments, experiment one with six steers in a three*three Latin square design and experiment two with four wether sheep in a cross over design, were conducted to study the effect of species and ammonia treatment on intake and utilization of the energy of untreated wheat straw. Treatments were: 1) untreated wheat straw offered ad libitum on top of a basal ration (B) consisting of hay (0.25) and grass pellets (0.75) (UWS), 2) ammoniated wheat straw fed ad libitum plus B (AWS) and 3) ammoniated wheat straw fed restricted plus B (AWS-). B was offered as a maintenance ration for both species and AWS- was only studied in steers. Voluntary intake of AWS was higher than that of UWS. No significant differences emerged between whole rations UWS and AWS with regard to energy digestion (ED), energy metabolizability (q = metabolizable energy (ME)/gross energy (GE)) and losses of digestible energy (DE) in urine and methane (average 187 J/kJ DE), but the efficiency of utilization of ME for growth (k_g) was significantly higher for AWS than for UWS. ED and q of the straw part of the ration was significantly higher for AWS than for UWS. AWS- and AWS did not differ significantly with regard to ED, q and DE losses in methane and urine. Steers had a higher intake per $kg^{0.75}$/day than wether sheep. Over species digestible energy intake (DEI) of the whole ad libitum fed rations was related to live weight $(W)^{0.946}$ (s.e. of exponent 0.0152). ED and q of the straw part of the rations did not differ significantly between species, but steers had a significantly higher ED and q of B than wether sheep. Steers excreted a significantly lower proportion of DE in urine and a significantly higher proportion of DE in methane than did wethers. Total energy losses in urine and methane, however, did not differ between species.

Keywords: *ammonia treatment, energy utilization, feed intake, steers, wethers, wheat straw.*

Introduction

Ammonia treatment of wheat straw is known to increase voluntary intake of digestible organic matter (DOMI) (Dias-da-Silva and Sundstøl, 1986), which is closely related to digestible energy intake (DEI) (Kellaway, 1969; Jeffery, 1971). Part of the DEI is lost as methane and urine. The Agricultural Research Council (ARC, 1980) accepted a value of 0.20 times DEI for total losses of DEI in methane and urine. Methane production is associated with production of acetate and butyrate in the rumen (Czerkawski, 1986). Since the rumen acetate production is relatively high for feeds with a high cell wall content such as wheat straw (Murphy, Baldwin and Koong, 1982), total losses of DEI in methane and urine could in case of wheat straw be higher than the constant applied by ARC (1980).
The digestible energy not lost in methane and urine can be metabolized by the animal and is called metabolizable energy (ME). The efficiency of utilization of ME for maintenance, growth or lactation is related to the metabolizability (q = ME/Gross Energy (GE)) of the feed and to ME intake level (ARC, 1980). The efficiency of ME utilization increases with increasing metabolizability and decreases with increasing MEI.

The experiments described here were designed to study the effect of ammonia treatment of wheat straw on voluntary intake, digestion, metabolizability and efficiency of utilization of energy.

DEI by cattle and sheep is assumed to be proportional to the maintenance requirements of the species (Blaxter, Wainman and Davidson, 1966; Weston, 1982). Since maintenance requirements per kg metabolic weight are higher for cattle than for sheep (ARC, 1980) scaling of digestible energy intake to liveweight $(W)^{0.9}$ may be more appropriate than scaling to $W^{0.75}$ when comparing sheep and cattle (Graham, 1972). Comparisons of digestible energy or organic matter intake of roughage by sheep and cattle, scaled to the fasting heat production (Blaxter *et al.*, 1966) or to $W^{0.9}$ (Poppi, Minson and Ternouth, 1981) did not show any systematic differences in intake capacity between the species. In a comparison of data from various experiments concerning DEI by sheep and cattle, Bird (1974) concluded that cattle are generally more efficient at deriving maintenance requirements from low-quality forages than are sheep. He related this species difference to the lower dietary sulphur requirements for cattle than for sheep.

The present paper describes a direct comparison of wether sheep and steers with regard to intake and utilization of energy from ammoniated and untreated wheat straw.

Material and methods

Experiment one

Steers and experimental design

Six steers (breed: Dutch red and white, a dual purpose breed) of approximately 16 months of age were kept on pasture with supplementary feeding of grass silage during winter until one month before the start of the first experimental period. From then they were kept in a tie stall and received the basal ration. During the experimental periods the animals were kept in tie stalls and open circuit respiration chambers (volume 10,000 l) during respectively three days and seven days to measure energy balances. The animals weighed on average 376 kg (s.e. 8.8) at the start and 408 kg (s.e. 7.4) at the end of the experiment.

The experiment consisted of two parts. During the first part (experimental period one) all animals received a basal ration (B) consisting of hay (0.25) and pelleted grass (0.75) offered at approximately maintenance level. During the second part of the experiment (experimental periods two up to four) the animals were randomly allocated to three treatments according to a 3*3 Latin square design with two animals per cell. Treatments were: 1) basal ration with chopped untreated wheat straw *ad libitum* (UWS), 2) basal ration with chopped ammonia-treated wheat straw at a restricted feeding level (AWS-) and 3) basal ration with chopped ammoniated wheat straw *ad libitum* (AWS). The amount of B offered during experimental periods two, three and four was similar to that in experimental period one. The quantity of straw offered in diet AWS- was as such, that MEI of this diet should be approximately similar to that of diet UWS. Expected MEI levels of AWS- and UWS were based on results of earlier experiments. The animals were fed twice daily at 07.30 and 16.30 h. The composition of the feeds is given in Table 1.

Before the start of the experiment the animals were familiarized with the respiration chambers. Adaptation periods between experimental periods lasted 17 days and experimental periods 10 days, during which residual feed, faeces and urine were collected. During the last seven days of each experimental period each steer was placed individually in a respiration chamber (temperature 20°C) in which heat production and methane production were measured once during 48 h and once or twice, depending on the repeatability of the results, for 24 h. Measurements started after three days of adaptation to the respiration chambers. Because only two respiration chambers were available, the steers were randomly divided into three groups of two animals. The experimental period started one week after the other for these three groups.

Table 1. Composition of diet constituents.

	Wheat straw	Ammoniated wheat straw	Pelleted grass	Hay
DM (g/kg)	902	900	931	874
OM (g/kg DM)	918	919	857	870
N (g/kg DM)	6	15	23	18
GE (MJ/kg DM)	17.7	17.8	17.3	16.7

Experiment two

Wethers and experimental design.

Four wether sheep (Swifter breed = Texel X Flemish) of approximately one year old were kept on pasture with supplementary feeding of hay during winter until one month before the first experimental period. From then the animals were housed individually in metabolism cages and received the basal ration. These metabolism cages were put in open circuit respiration chambers (volume 10,000 l) during part of the experimental period to measure energy balances. The animals weighed on average 46.6 kg (s.e. 1.50) at the start and 51.5 kg (s.e. 1.32) at the end of the experiment. Experiment two was conducted after experiment one.

The wethers were randomly allocated into two groups. During the first experimental period both groups received the same basal ration (B) as did the steers in experiment one also offered at approximately maintenance level. During the second and third experimental periods one group of wethers received the basal ration with chopped untreated wheat straw (UWS) and the other group the basal ration with chopped ammonia-treated wheat straw (AWS). The rations were crossed over from one group to the other after the second experimental period. During experimental periods two and three the amount of basal ration offered was equal to the amount offered during the first period and straw was offered *ad libitum*. The animals were fed twice daily at 07.30 and 16.30 h. The composition of the feeds is given in Table 1.

Before the start of the experiment the animals were familiarized with the respiration chambers. Adaptation periods lasted 17 days before experimental periods one and two and 10 days before period three. Faeces, urine and residual feed were collected during 10 days. During the last seven days of these experimental periods each sheep was placed individually in a respiration chamber (temperature 20°C), in which heat production and methane production were measured once during 48 h and once or twice, depending on the repeatability of measurements, for 24 h. Measurements started after three days of adaptation to the respiration chambers. The wethers were randomly divided into two groups of two

animals, because only two respiration chambers were available. The experimental period of these groups started one week after the other.

Ammonia treatment

The wheat straw was ammoniated in the late summer of 1990 by injecting 40 kg of anhydrous ammonia into stacks of 900 kg baled straw. The stacks were totally covered with two layers of polythene sheets of 0.15 mm thickness. During injection of the ammonia, tubes were inserted in the bottom part of the stacks to let air out. After all of the ammonia was injected into the stacks, these tubes were removed and the stacks were closed airtight. The stacks were opened after seven weeks. After two days of degassing the straw was chopped to a length of approximately 5 cm.

Analytical

In the various samples dry matter was determined at 103°C and organic matter by ashing at 550°C. N was measured according to the Kjeldahl method. Energy content of feed, faeces and urine were determined by adiabatic bomb calorimetry (IKA calorimeter C 4000). Methane was measured by gas chromatography (Packard 419; 6 m. stainless steel column filled with Porapak Q, 50-80 mesh at 60°C; carrier gas N_2; flame ionization detector 150°C; injection temperature 150°C). Heat production was calculated from oxygen consumption, carbon dioxide and methane production and urinary nitrogen excretion according to Brouwer (1965). The procedure of O_2 and CO_2 analysis was described in detail by Van Es (1958).

Calculations and statistics

DEI was equal to gross energy intake (GEI) minus faecal energy. Energy digestion (ED) was defined as DEI/GEI. Metabolizable energy intake (MEI) was calculated by subtracting energy in urine and methane from DEI. The q value was defined as MEI/GEI. The energy balance (EB) was assessed by subtracting heat production from MEI. GEI, DEI and MEI of the straw part of the ration were calculated as the difference between the total intake and the intake of the basal feed. This was based on the assumption that no interaction existed between basal diet and straw with regard to ED and q and, in addition, absence of a period effect on ED or q of the basal ration. The latter assumption had to be made, because the experimental design did not allow for testing of a period effect on ED and q of B. The assumption that no interactions were present between basal feed and straw with regard to ED and q was tested for ammonia-treated wheat straw-based rations in steers by comparison of diets B, AWS- and AWS by the following regression model:
Y = intercept + b_1*OMI_{straw} + b_2*OMI_{straw}^2. Y is either organic matter digestion (OMD), ED or q, OMI_{straw} is OMI of AWS (zero in the case of ration B) and b_1 and b_2 are the linear

and quadratic regression coefficient, respectively. Curvilinearity of the relation between Y and OMI_{straw} is an indication of interaction between basal feed and straw. This analysis was based on the assumption, that a period effect on OMD, ED or q of B was not present.

Estimation of the exponent b of liveweight (W, kg) at which $DOMI/W^b$ or DEI/W^b were equal for both species was done by the following model (the logarithmic transformation of the general model $Y = a*W^b$) in the pooled data set of experiments one and two for rations UWS and AWS:
$\ln(Y) = \mu + ration_i + (b + \Delta b_i)*\ln(W)$, with b as the overall exponent and Δb_i as the difference between the overall and within-ration exponent of W to which DOMI or DEI (Y, kg or kJ) were related across species.

Analysis of variance of separate data sets of experiments one (experimental periods two up to four) and two (experimental periods two and three) was done by the model $Y_{ijkl} = \mu + ration_i + animal_j + period_k + error_{ijkl}$. Results of first experimental periods of both experiments were analysed by the model $Y_{ij} = \mu + species_i + error_{ij}$ to test the species effect on digestion and metabolizability of diet B.

Species effect and differences between UWS and AWS were analysed in the pooled data set of experiments one and two for the rations UWS and AWS by the model $Y_{ijk} = \mu + ration_i + species_j + ration_i*species_j + error_{ijk}$. Analysis of variance and testing of significance of differences between means by Student's t-tests was done by the statistical program DBSTAT (Brouwer, 1990).

Results

Comparison of intake, digestion and utilization of energy by sheep and cattle is only relevant, if the feeding history and the physiological age of both species are comparable (Weston, 1982). The steers and wether sheep in the present experiments had a comparable feeding history. Both species had been grazing pasture with some supplementary feeding of hay (wethers) or grass silage (steers) during winter and all animals were fed ration B during the last month before the start of the experiment. The physiological age could be compared by expressing the weight as a proportion of the adult weight of females. The average adult weight of Dutch red and white females is approximately 650 kg and the adult weight of Swifter ewes approximately 80 kg. The average weight of the species as a proportion of the average adult weight of females was thus 0.60 and 0.61 for the steers and wether sheep, respectively.

Basal diet

Data for intake, digestion, q and EB for steers and wethers fed B alone during the first experimental period of both experiments is presented in Table 2. B should be offered

at maintenance level. Because steers have higher maintenance requirements than wethers (ARC, 1980), the energy offered through B was more for steers than for wethers. For both steers and wethers, digestion of B was, however, lower than could reasonably be assumed before the experiment. This meant, that wethers were unable to meet their maintenance requirements as illustrated by the negative energy balance. Steers could, however, maintain themselves on B.

Steers digested energy and organic matter (OM) from the basal ration significantly better than wethers and also the q value was significantly higher for steers than for wethers. Total losses of DE in urine and methane were slightly, though significantly different between wethers and steers, because steers excreted significantly less energy as a proportion of DE in urine than did wethers.

Table 2. Intake and digestion of OM and energy, metabolizability of energy, energy balance and average weight for steers and wethers fed B alone. (Between brackets s.e.m.)

	Wethers (n = 4)		Steers (n = 6)	
OMI ($g/kg^{0.75}$/day)	32.4^a	(0.35)	37.3^b	(0.25)
OMD (g/kg)	582^a	(8.7)	630^b	(7.1)
DOMI ($g/kg^{0.75}$/day)	18.8^a	(0.24)	23.5^b	(0.20)
Intake ($kJ/kg^{0.75}$/day)				
- GE	652^a	(6.11)	750^b	(5.00)
- DE	348^a	(5.40)	435^b	(4.40)
- ME	278^a	(4.53)	356^b	(3.70)
ED (J/kJ)	535^a	(9.2)	580^b	(7.6)
q (ME/GE)	0.427^a	(0.0079)	0.476^b	(0.0064)
Losses of DE in:				
- methane (J/kJ DE)	115	(4.6)	112	(3.7)
- urine (J/kJ DE)	89^a	(1.6)	69^b	(1.3)
- total (J/kJ DE)	204^b	(4.8)	181^a	(3.9)
Energy balance ($kJ/kg^{0.75}$/day.)	-71.5^a	(7.39)	3.6^b	(6.03)
Weight (kg)	46.8^a	(8.55)	377.7^b	(6.98)

Different superscripts per row indicate significant difference ($P < 0.05$)

Table 3. Intake and digestion of organic matter of rations consisting of B and straw and average weight of animals.

	Wethers (n = 4)			Steers (n = 6)				Statistics pooled data'		
	UWS	AWS	s.e.m.	UWS	AWS	AWS-	s.e.m.	Ration	Species	Ration * species
OMI (g/kg$^{0.75}$/day)										
- *whole ration*	45.2^{a}	51.6^{b}	1.29	66.3^{b}	76.5^{c}	57.2^{a}	0.78	***	***	NS
- *straw*	14.9^{a}	21.4^{b}	1.54	29.8^{b}	40.1^{c}	20.6^{a}	0.78	***	***	NS
OMD (g/kg)										
- *whole ration*	528	545	9.5	546^{a}	561^{a}	591^{b}	6.3	NS	*	NS
- *straw*	414	495	22.9	443^{a}	498^{b}	534^{b}	13.9	**	NS	NS
DOMI (g/kg$^{0.75}$/day)										
- *whole ration*	23.9^{a}	28.1^{b}	0.36	36.2^{b}	43.0^{c}	34.0^{a}	0.67	***	***	NS
- *straw*	6.2^{a}	10.5^{b}	0.53	13.3^{b}	20.0^{c}	11.0^{a}	0.68	***	***	NS
Weight (kg)	51.0	51.3	0.64	387.7^{a}	389.9^{b}	387.7^{a}	0.33			

Different superscripts per row per species indicate significant differences.
*: P < 0.05; **: P < 0.01; ***: P < 0.001; NS: not significant.
' Data AWS- not included.

Whole rations and straw

Organic matter intake (OMI), organic matter digestion (OMD) and digestible OMI (DOMI) of the whole rations UWS, AWS and AWS- and straw components of these rations are given in Table 3. Within and across species, OMI of whole rations (OMI_{wr}), $DOMI_{wr}$, OMI of the straw component (OMI_{straw}) and $DOMI_{straw}$ were significantly higher for AWS than for UWS. No significant differences emerged between UWS and AWS for OMD_{wr}, while OMD_{straw} was significantly higher for AWS than for UWS in steers and across species. In steers, OMD_{wr} was significantly higher and OMD_{straw} tended ($P < 0.10$) to be higher for AWS- than for AWS. Regression of OMD_{wr} on OMI_{straw} for rations B, AWS- and AWS in steers to test for an interaction between basal diet and ammoniated wheat straw resulted in the following linear relationship (between brackets, s.e. of estimate):
$OMD_{wr} = 629\ (6.3) - 1.7\ (0.24) * OMI_{straw}$, $R^2 = 0.747$, RSD = 16.9, n = 18. A quadratic component was not significant, indicating, that no interaction was present between basal diet and ammoniated wheat straw with regard to OMD.

Steers had higher OMI_{wr}, $DOMI_{wr}$, OMI_{straw} and $DOMI_{straw}$ of UWS and AWS than wethers, if intake was scaled to $W^{0.75}$. Across species $DOMI_{wr}$ and $DOMI_{straw}$ of *ad libitum* fed rations were related to $W^{0.957}$ (s.e. of exponent 0.0152) and $W^{1.10}$ (s.e. of exponent 0.0485), respectively. The values of the exponents were not significantly different between rations UWS and AWS. Both exponents were significantly higher than 0.9. DOMI of B as component of the whole *ad libitum* fed rations was related to $W^{0.884}$ (s.e. of exponent 0.0082). Steers and wethers did not differ significantly with regard to OMD_{straw} of rations UWS and AWS, but OMD_{wr} of these rations was significantly higher for steers than for wethers.

Data for energy intake, ED, q and EB for whole rations and for straw components of whole rations are given in Table 4. Within and across species intake of GE, DE and ME of whole rations and of straw components was significantly higher for AWS than for UWS. No significant differences in ED_{wr} and q_{wr} were observed between UWS and AWS. ED_{straw} and q_{straw} of AWS were significantly higher than of UWS, although only a tendency ($P < 0.10$) existed in the case of wethers. In steers, AWS- had a significantly higher ED_{wr} and q_{wr} than the other rations. The higher ED_{wr} and q_{wr} of AWS- compared with AWS was mainly caused by the higher proportion of B in this ration. ED_{straw} and q_{straw} were not significantly higher for AWS- than for AWS. The relationships between ED_{wr} or q_{wr} and OMI_{straw} were linear (analysis on diets B, AWS- and AWS in steers), indicating absence of interactions between basal diet and ammoniated wheat straw in steers. The following relationships were obtained (between brackets, s.e. of estimate): $ED_{wr} = 580\ (5.8) - 1.5\ (0.22) * OMI_{straw}$, $R^2 = 0.736$, RSD = 15.7, n = 18 and $q_{wr} = 474\ (5.7) - 1.3\ (0.22) * OMI_{straw}$, $R^2 = 0.702$, RSD = 15.3, n = 18.

Across and within species the energy balance was significantly higher for AWS than for UWS. The proportion of DE losses in methane energy did not differ significantly between rations UWS and AWS. In steers, urinary energy losses as a proportion of DE were slightly,

Table 4. Intake, digestion and metabolizability of energy and energy balance of rations consisting of B and straw.

	Wethers (n = 4)			Steers (n = 6)				Statistics pooled data[†]		
	UWS	AWS	s.e.m.	UWS	AWS	AWS-	s.e.m.	Ration	Species	Ration * species
Intake (kJ/kg$^{0.75}$/day)										
GE										
- *whole ration*	905^{a}	1049^{b}	21.9	1307^{b}	1511^{c}	1135^{a}	15.2	***	***	NS
- *straw*	294^{a}	440^{b}	25.8	577^{b}	785^{c}	405^{a}	15.2	***	***	NS
DE										
- *whole ration*	438^{a}	534^{b}	5.2	662^{b}	785^{c}	623^{a}	11.1	***	***	NS
- *straw*	111^{a}	209^{b}	8.2	241^{b}	365^{c}	200^{a}	10.7	***	***	NS
ME										
- *whole ration*	358^{a}	437^{b}	4.2	537^{b}	636^{c}	504^{a}	9.9	***	***	NS
- *straw*	91^{a}	171^{b}	6.0	191^{b}	291^{c}	157^{a}	9.7	***	***	NS
ED (J/kJ)										
- *whole ration*	483	510	10.9	507^{a}	519^{a}	550^{b}	5.2	NS	NS	NS
- *straw*	367	479	25.2	418^{a}	464^{b}	494^{b}	12.3	**	NS	NS
q (ME/GE)										
- *whole ration*	0.395	0.417	0.0119	0.411^{a}	0.420^{a}	0.444^{b}	0.0051	NS	NS	NS
- *straw*	0.299	0.392	0.0222	0.330^{a}	0.370^{b}	0.389^{b}	0.0111	**	NS	NS
Energy losses (J/kJ DE):										
- *methane*	115	111	3.6	136	131	131	3.6	NS	***	NS
- *urine*	69	71	3.5	54^{a}	59^{b}	60^{b}	1.1	NS	***	NS
- *total*	183	182	5.0	190	190	191	3.8	NS	NS	NS
Energy balance (kJ/kg$^{0.75}$/day)	-35.8^{a}	28.0^{b}	2.55	28.0^{a}	87.2^{b}	31.8^{a}	7.74	**	**	NS

Different superscripts per row per species indicate significant differences.
**: P < 0.01; ***: P < 0.001; NS: not significant. [†] Data AWS- not included.

though significantly higher for AWS and AWS- than for UWS, while no significant difference between rations was observed in wethers. Total DE losses in methane and urine did not differ between rations. Urinary and methane energy losses as a proportion of DE were negatively correlated ($r = -0.80$, $n = 26$, $p < 0.001$).

Steers consumed significantly more of UWS and AWS than did wethers, if intake was scaled to $W^{0.75}$. Estimates of the exponent of W, to which DEI was related across species were 0.946 (s.e. 0.0152) and 1.092 (s.e. 0.0485) for whole rations UWS and AWS and straw components of these rations, respectively. The values of the exponents were not significantly different between rations. Both exponents were significantly higher than 0.9. DEI of B as a component of UWS and AWS was related to $W^{0.875}$ (s.e. of exponent 0.0075).

No significant differences were observed between species with regard to ED_{straw}, q_{wr} and q_{straw}. ED_{wr} of UWS and AWS tended ($P < 0.10$) to be higher for steers than for wethers. Steers had significantly higher methane losses, but significantly lower urinary energy losses as a proportion of DE than did wethers. As a result, total DE losses in methane and urine were not different between species. The energy balance of steers fed UWS or AWS was significantly higher than that of wethers.

Discussion and conclusions

Maintenance requirements and efficiency of ME utilization

Net energy requirements for maintenance (NE_m) given in the literature for yearling wether sheep are in the range of 200 to 275 kJ/kg$^{0.75}$/day (Rattray, Garrett, Hinman, Garcia and Castillo, 1973; Graham, Searle and Griffiths, 1974; Blaxter, 1989; Ketelaars and Tolkamp, 1991). For steers of approximately 400 kg reported NE_m values are in the range of 235 to 370 kJ/kg$^{0.75}$/day (Moe, Tyrrell and Flatt, 1970; ARC, 1980; Blaxter, 1989). The large variation in NE_m within species is caused by breed differences and variation in feeding history. Beef breeds have lower NE_m than dairy breeds (Blaxter, 1989) and animals may adapt to low energy intake by reducing their NE_m (Graham *et al.*, 1974; Shetty, 1990). The wide range of possible NE_m within species means that no average value could be adopted from literature. NE_m, ME_m (maintenance requirements for ME) and the efficiency of ME utilization for growth (k_g) or for growth and maintenance (k_{m+g}) were therefore estimated by two methods.

1) ARC (1980) gives the following formula to relate k_m to q_m (the q value at maintenance): $k_m = 0.35*q_m + 0.503$. For ration B (assuming that the observed q was equal to q_m) estimates of k_m were 0.67 for steers and 0.65 for wethers. From $NEI = k_m*MEI$ and $NE_m = NEI - EB$, NE_m for steers and wethers were calculated as 235 and 250 kJ/kg$^{0.75}$/day, respectively. Estimates of k_m (derived from the ARC formula) for rations AWS in steers and

Table 5. Efficiency of ME utilization for growth (k_g) and for maintenance and growth (k_{m+g}). Alternative 1: NE_m 235 kJ/kg$^{0.75}$/day for steers and 250 kJ/kg$^{0.75}$/day for wethers, k_m for all rations 0.65. Alternative 2: NE_m 288 kJ/kg$^{0.75}$/day for steers and 245 kJ/kg$^{0.75}$/day for wethers, k_m for all rations 0.65.

	Wethers (n = 4)			Steers (n = 6)				Statistics pooled data[†]		
	UWS	AWS	s.e.m.	UWS	AWS	AWS-	s.e.m.	Ration	Species	Ration * species
Alternative 1:										
k_g	-	0.43	0.208	0.16[a]	0.31[b]	0.22[a]	0.022	-	-	-
k_{m+g}	0.60	0.64	0.007	0.49[a]	0.51[a]	0.53[b]	0.006	*	***	NS
Alternative 2:										
k_g	-	0.47	0.173	0.28[a]	0.44[b]	0.49[b]	0.038	-	-	-
k_{m+g}	0.59	0.62	0.007	0.59[a]	0.59[a]	0.63[b]	0.006	NS	NS	NS

Different superscripts per row per species indicate significant differences.
**: P < 0.01; ***: P < 0.001; NS: not significant.
[†] Data AWS- not included.
- not available

wethers and AWS- and UWS in steers were all 0.65. ME_m for these rations (NE_m/k_m) was therefore 362 and 385 kJ/kg$^{0.75}$/day for steers and wethers, respectively. Table 5 (alternative 1) gives the estimates for k_g for the rations with a positive EB and k_{m+g} for all rations. The k_g was calculated as EB/(MEI-ME_m) and k_{m+g} as (NE_m + EB)/MEI. In wethers, the k_{m+g} for UWS is equal to k_m. In steers, AWS- and UWS had a significantly lower k_g than did AWS. The k_{m+g} tended (P = 0.09) to be higher for AWS than for UWS in wethers and was significantly higher for AWS- than for the other rations in steers.

ARC (1980) and Blaxter (1989) proposed a curvilinear relationship between EB and MEI, implying a reducing k_g with increasing MEI of the same feed. B as a proportion of the total ration was higher for AWS- than for AWS, which resulted in a significantly higher q_{wr} value for AWS- than for AWS. For these two reasons it was expected that AWS- would at least have a similar k_g to that of AWS. It seems therefore likely, that estimation of NE_m from the observed EB for B resulted in an underestimation in the case of steers. Therefore an alternative method was applied to calculate NE_m values.

2) By accepting a linear relationship between EB and MEI for AWS- and AWS in steers, regression of EB on MEI for these two rations gives an estimate of ME_m and k_g. The regression equation obtained was (between brackets, s.e. of estimate):

EB (kJ/kg$^{0.75}$/day) = -208 (31.1) + 0.47 (0.054) * MEI (kJ/kg$^{0.75}$/day) (R^2 = 0.882, n = 12, RSD = 13.6).

This equation implies a k_g of 0.47 and an ME_m of 440 kJ/kg$^{0.75}$/day. The latter corresponds to an NE_m (based on k_m = 0.65) of 288 kJ/kg$^{0.75}$/day. There are no indications from the literature (ARC, 1980; Blaxter, 1989), that sheep and cattle differ with regard to k_g values. Applying a k_g of 0.47 to the average EB observed for AWS fed to wether sheep resulted in an estimate of ME_m of 377 and of NE_m of 245 kJ/kg$^{0.75}$/day. Data and the regression line for EB on MEI for rations AWS- and AWS in steers are plotted in Figure 1.

Estimates of k_g and k_{m+g} for the various rations based on these maintenance requirements are given in Table 5. In steers, AWS and AWS- had a significantly higher k_g than UWS. Estimates for k_{m+g} for *ad libitum* rations were almost similar, though k_{m+g} tended (P = 0.06) to be higher for AWS than for UWS in sheep. Provided that the maintenance requirements accepted for estimation of these k_{m+g} values were correct and that these results are generally applicable, for practical purposes the NE content of an *ad libitum* fed ration could be estimated as 0.6 times its ME content. Tolkamp and Ketelaars (1993) arrived at a similar conclusion, when they calculated k_{m+g} from models given by ARC (1980) for *ad libitum* fed growing cattle on two diet types with q_m values ranging from 0.45-0.65.

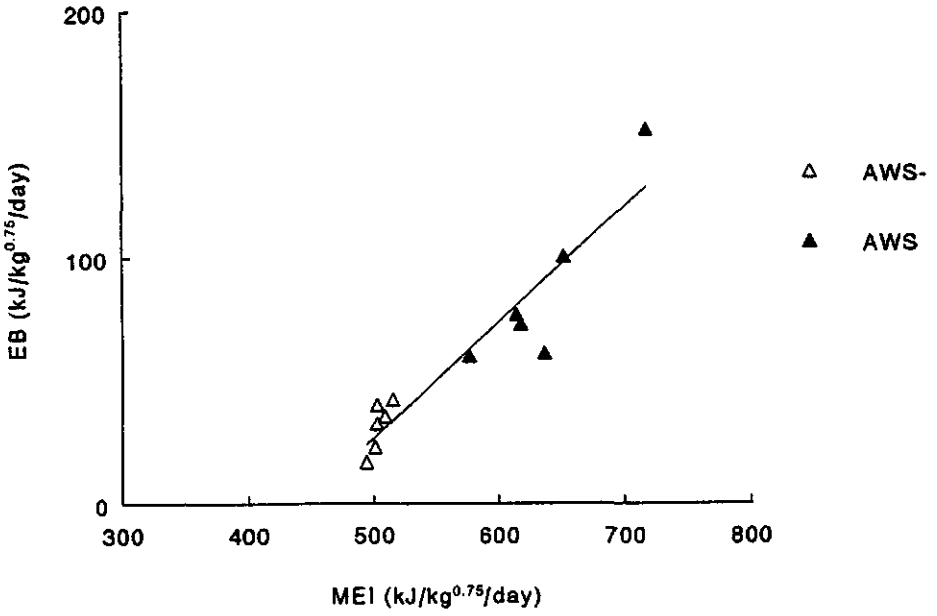

Figure 1. Relationship between EB and MEI for ammonia-treated wheat straw based rations in steers.

In conclusion, estimates of maintenance requirements from both methods of estimation were approximately similar for wether sheep, but differed considerably for steers. Why this discrepancy was observed for steers is unknown. Both methods of estimation resulted in higher estimates of k_g for AWS than for UWS. The q_{wr} of UWS and AWS were approximately similar and the different k_g values observed between AWS and UWS could therefore not be related to quality differences between these rations. It is likely, that UWS should be regarded as being in a different feed class from that of AWS. ARC (1980) gave relations between k_f (k_g at twice maintenance) and q_m, which differ between classes of feeds. Both alternatives given above are based on the assumption that the ARC equations are applicable to the diets fed in the present experiment. Estimates of k_m for feeds with a q_m ranging from 0.4 to 0.5 in various feed classes as given by ARC (1980) are close to 0.65. If, however, assumptions about a more or less constant k_m of 0.65 are in error then obtained estimates of NE_m are erroneous.

Species comparison

Across species both DEI_{wr} and DEI_{straw} were related to W to an exponent significantly higher than 0.9. Maintenance requirements of sheep and cattle are, across species, related to $W^{0.9}$ (Graham, 1972). DEI_{wr} and DEI_{straw} were scaled to NE_m, accepting NE_m values of 245 and 288 $kJ/kg^{0.75}$/day for wether sheep and steers, respectively. Averaged over rations UWS and AWS, DEI_{wr} was 1.98 and 2.51 (s.e.m. 0.045) times NE_m and DEI_{straw} 0.65 and 1.05 times NE_m (s.e.m. 0.053) for wethers and steers, respectively. Both DEI_{wr} and DEI_{straw} scaled to NE_m were significantly higher for steers than for wethers. ARC (1980) proposed a value of 320 $kJ/kg^{0.75}$/day as NE_m for cattle. If for steers DEI_{wr} and DEI_{straw} were scaled to this value estimates were 2.26 and 0.95 times NE_m, respectively. Also these values were significantly higher than those for wethers. The conclusion seems therefore justified that, proportionally to their maintenance requirements, steers had a higher voluntary DEI than wethers. This is in contrast with observations of Blaxter *et al.* (1966) and Poppi *et al.* (1981), who found that voluntary DEI of cattle and sheep was proportional to maintenance requirements across species. DEI of B scaled to NE_m of 245 $kJ/kg^{0.75}$/day for wethers and 288 $kJ/kg^{0.75}$/day for steers was 1.33 and 1.46 for wethers and steers, respectively. It seems unlikely that this rather small difference in DEI from B could be the cause of the much greater difference in DEI_{straw} between species. ED_{straw} and q_{straw} did not differ between species and consequently could not explain differences in intake between species. Why steers digested B significantly better than wethers, while no difference between species with regard to ED_{straw} was observed, is unknown.

Bird (1974) observed that cattle had higher DEI scaled to $W^{0.92}$ than sheep for wheat straw supplemented with urea, but not for unsupplemented wheat straw or wheat straw supplemented with urea and sulphur. He therefore suggested that differences in dietary sulphur requirements contributed to the species difference with regard to DEI for diets with a wide nitrogen to sulphur ratio. Sulphur availability relative to N availability from the rations in the present experiment could be limiting DEI of wether sheep more than that of steers. This would imply that, compared with UWS, DEI of AWS should be higher for steers than for wethers, because the ammonia-N added through the treatment widened the nitrogen to sulphur ratio in the ration. Scaled to maintenance requirements of 245 and 288 kJ $NE/kg^{0.75}$/day DEI of UWS was 1.79 and 2.30 times NE_m for wethers and steers, respectively. DEI of AWS was 2.18 and 2.73 times NE_m for wethers and steers, respectively. DEI of AWS minus DEI of UWS was only slightly higher for steers than for wethers (0.43 and 0.39 times NE_m, respectively). The absence of a difference between wethers and steers could not be attributed to a difference in sulphur supply through the basal ration. The basal rations of both species were identical and had therefore a similar sulphur/GE ratio. GEI from B as part of the whole rations did not differ between species (2.49 and 2.53 times NE_m for wethers and steers, respectively) and consequently sulphur supply, scaled to maintenance requirements, had not differed either. The conclusion seems therefore justified that dietary sulphur availability relative to requirements did not cause the differences in DEI scaled to

NE_m between species.

Methane losses as a proportion of DEI were significantly higher for steers than for wethers for straw-based rations, but not when B was fed alone. Methane production is associated with production of acetate and butyrate in the rumen (Czerkawski, 1986). The proportion of acetate in the total volatile fatty acid production is negatively correlated with the cell wall content of ingested feed (Murphy *et al.*, 1982). Thus the higher proportion of straw in the rations fed to steers compared with wethers could partly explain the difference in methane losses as a proportion of DEI between species. However, calculation of methane losses attributable to straw yielded estimates of 112 and 170 J/kJ DEI for wethers and steers, respectively. A possible explanation is that the contribution of fermentation in the hindgut to whole tract digestion was higher in wethers than in steers. Less methane is produced per unit energy fermented in the hindgut than in the rumen (Demeyer, 1991). Another reason, however, could be that steers had a different type of fermentation in the rumen resulting in higher proportions of acetate and/or butyrate in the total volatile fatty acids. Whether differences in fermentation between wethers and steers could explain the differences in intake is unknown. If fermentation in steers was associated with a higher efficiency of microbial protein synthesis compared with wethers, the protein/energy ratio in the digestion products could be higher for steers than for wethers, which may affect voluntary intake positively (Egan, 1977).

Steers excreted a significantly lower proportion of their DEI as urinary energy than did wethers. Urinary excretion of energy is related to excretion of N-containing products, which is related to the N and energy balance. Steers had a higher energy balance than wethers, and as a consequence probably a higher N retention, but even at comparable levels of energy balance (AWS in wethers and AWS- in steers) wethers lost a higher proportion of their DEI as urinary energy than did steers. Total methane and urinary energy losses as a proportion of DEI were not significantly different between steers and wethers.

Ration comparison

Ammoniation of wheat straw increased intake relatively more than it increased digestion and metabolizability. Across species GEI_{straw} increased proportionally by 0.43, while ED_{straw} and q_{straw} increased by 0.21 owing to ammonia treatment of the wheat straw. A higher effect of ammonia treatment on intake of straw than on digestion was also found by Dias-da-Silva and Sundstøl (1986) and Silva, Greenhalgh and Ørskov (1989). DEI_{straw} and MEI_{straw} increased proportionally by 0.70 (average for both species) after ammonia treatment.
No differences in methane energy losses as a proportion of DEI were found between UWS and AWS. In steers, UWS had slightly, but significantly lower urinary energy losses as a proportion of DEI than AWS, probably because of a lower urea excretion. Differences in ED_{wr} and q_{wr} between AWS and AWS- fed to steers could mainly be attributed to a higher proportion of B in AWS- than in AWS. ED_{straw} and q_{straw} of AWS- were only slightly, and insignificantly higher than of those of AWS. A higher ED_{straw} and q_{straw} for AWS- compared

with AWS could be expected based on the lower intake level (ARC, 1980). The slightly higher ED_{straw} and q_{straw} values found in steers for AWS- compared with AWS are therefore probably not indications of interactions between the basal diet and ammoniated wheat straw. The latter was supported by the fact, that relations between OMD_{wr}, ED_{wr} or q_{wr} and OMI_{straw} were linear. Interactions between basal diet and straw, however, could not be tested for wethers and for UWS in steers.

In conclusion, the results of the present experiments showed, that voluntary DEI of straw-based rations scaled to maintenance requirements was higher for steers than for wethers. Species did not differ with regard to ED and q of whole straw-based rations and of straw, but steers digested energy from the basal diet significantly better than did wethers. Ammoniation of wheat straw positively affected voluntary intake, digestion and metabolizability and the efficiency of utilization of energy. The losses of DEI in urinary energy and methane energy were not affected by species or ration and were on average 187 J/kJ DEI.

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CHAPTER 4.

PROTEIN SUPPLEMENTATION TO AMMONIATED WHEAT STRAW

4.1 Protein supplementation to ammoniated wheat straw. 1. Intake, digestion, rumen fermentation and passage characteristics in sheep

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Abstract

The effects of supplementation of protein sources of either a high (casein) or a relatively low rumen degradability (potato protein) on intake and digestion of ammoniated wheat straw were studied with four sheep in a 4x4 Latin square design. Rations offered were: 1) untreated wheat straw (UWS), 2) ammoniated wheat straw (AWS), 3) AWS supplemented with 3.2 g/kg$^{0.75}$/day of casein (AWSC) and 4) AWS supplemented with 3.9 g/kg$^{0.75}$/day of potato protein (AWSP). Straw was offered *ad libitum* and all rations were supplemented with sugar beet pulp and a mineral mixture. Digestible organic matter intake (DOMI) of ammoniated wheat straw was higher ($P < 0.05$) for AWSP than for AWSC. Compared with AWS, potato protein supplementation increased ($P > 0.05$) DOMI of ammoniated wheat straw, whereas DOMI of ammoniated wheat straw was substituted by that of casein ($P < 0.05$). Protein supplementation to ammoniated wheat straw reduced rumen degradation of cell wall components (casein: $P < 0.05$ for NDF, hemicellulose and cellulose; potato protein: $P < 0.05$ for cellulose). This lower rumen degradation was compensated by a higher partial digestion in the hindgut for hemicellulose ($P < 0.05$ for AWSC, $P > 0.05$ for AWSP), but not for cellulose, resulting in a significantly decreased whole-tract digestion of cellulose for AWSP and AWSC compared with AWS. Rumen pH and volatile fatty acid (VFA) concentration were not significantly affected by protein supplementation, but casein ($P < 0.05$) and potato protein ($P > 0.05$) supplementation increased rumen ammonia-N concentration. The rate of passage from the rumen of fluid and particulate phases and the rate of absorption of VFA's were not significantly affected by protein supplementation. Intake and digestion of wheat straw increased significantly as a result of ammonia treatment. Ammoniation of wheat straw had no significant effect on contribution of the rumen to whole tract digestion, rumen pH and VFA concentration, rate of passage of fluid and particulate phases and rate of absorption of VFA's. The rumen fluid volume and the rumen ammonia-N concentration were significantly higher for AWS than for UWS. Across all rations, rumen fluid volume increased with increasing cell wall intake. The rumen was the most important site for cell wall digestion. The average contribution of the rumen to whole tract NDF digestion was 90.8 %, without significant differences between rations.

Introduction

An experiment with untreated and ammonia treated wheat straw fed to sheep (Van Bruchem et al., 1993a; Oosting et al., 1993) indicated that, although ammoniation of wheat straw resulted in a considerable increase of energy intake, the availability of amino acids for absorption from the small intestine remained quite low. The latter was due to a relatively low extent and efficiency of microbial protein synthesis in the rumen and a low quantity of undegraded dietary protein passing from the rumen. Supplementation of proteins to ammonia-treated wheat straw could increase the efficiency of microbial protein synthesis in the rumen by supplying nutrients potentially limiting microbial growth as oligo-peptides, branched chain amino acids or minerals (Hoover, 1986). In case the protein supplement has a low rumen degradability and high small intestinal true digestibility, the small intestinal availability of amino acids increases as a result of an increased flow of undegraded dietary protein from the rumen.

An increased small intestinal availability of amino acids may result in a higher roughage intake (Egan and Moir, 1965; Egan, 1977; Preston and Leng, 1987), although this could not be confirmed in several other experiments (Kellaway and Leibholz, 1983; Ketelaars and Tolkamp, 1991).

The present experiment was conducted to study the effect of supplementation of proteins with a low and high rumen degradability to ammonia-treated wheat straw on intake, digestion and site of digestion, rumen fermentation and small intestinal amino acid availability. The latter is reported elsewhere (Van Bruchem et al., 1993b).

Materials and methods

Four sheep (wethers, breed Swifter, a cross-bred of Texel and Flemish) with an average liveweight of approximately 61 kg were fitted with a cannula in the dorsal rumen sac (hard PVC, 25 mm internal diameter), and with T-shape hard PVC cannulas (12 mm i.d.) in the proximal duodenum and terminal ileum. The experiment started approximately two months after surgery, after the animals had well recovered. During the experiment the animals were kept in metabolism cages and received equal portions of their ration every four hours starting at 00.00 h. Water was freely available.

The experiment was set up as a 4 x 4 Latin square design. The four diets that were tested were:

1) untreated wheat straw (UWS),
2) ammoniated wheat straw (AWS),
3) ammoniated wheat straw supplemented with casein (DMV-Campina, Veghel) (AWSC)
4) ammoniated wheat straw supplemented with potato protein (Emsland-Stärke GmbH, Emlichheim, Germany) (AWSP).

Straw was fed *ad libitum*, by offering at least 25 % excess. All diets were supplemented with sugar beet pulp, minerals and a commercially available mixture of vitamines A and D_3 and trace elements (Mervit 318; Premervo, Utrecht, The Netherlands). Sugar beet pulp, minerals and vitamines and, for diets AWSC and AWSP, the protein supplements were mixed and offered as a pellet. The proportions of constituents in the supplements without additional protein (SBP), with additional casein (SBPC) and with additional potato protein (SBPP) are given in Table 1. Sugar beet pulp was offered at a level of 16.7 g DM/$kg^{0.75}$/day and the quantity of the mineral/vitamin mixture offered was 1.8 g DM/$kg^{0.75}$/day. Casein and potato protein were given additionally at levels of 3.2 and 3.9 g/$kg^{0.75}$/day, respectively. The supplementary casein and potato protein were iso-nitrogenous. The chemical composition of the ration components is given in Table 2.

Table 1. Proportions of constituents (g/kg) in supplements sugar beet pulp + minerals and vitamines (SBP), SBP + casein (SBPC) and SBP + potato protein (SBPP).

	SBP	SBPC	SBPP
Sugar beet pulp	904	742	713
Mervit 318 (Vit. A and D_3, trace elements)	45	37	36
$NaH_2PO_4.2H_2O$	32	26	25
$FeSO_4.7H_2O$	0.5	0.4	0.4
$MgSO_4.7H_2O$	19	15.6	15
Additional protein	nil	178	211

Table 2. Chemical composition (DM: g/kg product, all other fractions: g/kg DM) of ration components.

	Untreated wheat straw	Ammoniated wheat straw	SBP	SBPC	SBPP
DM	910	905	848	816	840
Ash	81	84	94	79	86
N	6	14	15	39	37
NDF	781	758	395	342	361
Hemicellulose	289	263	171	161	176
Cellulose	425	439	204	161	164
Lignin	66	55	20	20	20

Table 3. Time chart of activities during an experimental period.

	Experimental week[1]						
	1111111	2222222	3333333	4444444	5555555	6666666	7777777
Adaptation	*******	*******	*****				
Dosing of marker			****	******		***	******
Rumen fluid samples:							
-Co (rumen fluid volume)				*****		*****	
-Co (k_1), NH_3, VFA, pH				**		**	
Faecal excretion Cr				*	*****	*	*****
Flow small intestine				****		****	
Intake and digestion			**	*******	***	** *******	***

1 days within weeks: Sunday up to Saturday

The wheat straw was ammoniated in the late summer of 1990 by injecting 40 kg of anhydrous ammonia into stacks of approximately 900 kg (dry matter basis) baled wheat straw. The stacks were covered air-tight with two layers of polythene sheets of 0.15 mm thickness. During injection, tubes were inserted in the bottom of the stacks to let air out. These tubes were removed immediately after injection of the ammonia. The stacks were opened after 35 days. After degassing for two days the straw was chopped to a length of approximately 5 cm. Untreated wheat straw was chopped to the same size.

Before the start of the experiment the animals had well adapted to the straw rations and to the experimental routine. Each of the four experimental periods had a duration of seven weeks. The time chart of measurements over an experimental period is given in Table 3. The first three weeks were adaptation period. During the third and fifth week Cr mordanted NDF (Cr-NDF, average Cr concentration 53 g/kg) and Co-EDTA (average Co concentration 148 g/kg), both prepared according to Udén et al. (1980) were introduced into the rumen at intervals of 6 h to get a steady state concentration of Cr and Co in the rumen. Dosing of Cr and Co continued during the first four days of weeks four and six up to Friday morning 06.00 h. Dosing was done at 6.00, 12.00, 18.00 and 24.00 h. The daily amounts introduced into the rumen of Cr-NDF and Co-EDTA were 10 g and 3 g, respectively, which resulted in average daily duodenal and ileal flows of Cr and Co of 527 and 443 mg, respectively.

The concentration of Co in rumen fluid was measured in samples of 50 ml taken on Monday up to Thursday in weeks four and six, at 08.00 and 10.00 h. The rumen fluid volume (RFV) was estimated from the Co concentration in the rumen fluid samples by the following equation, applying to a steady-state situation in the rumen:

RFV (l) * concentration of Co (mg/l) * k_l (/day) = daily dosage of Co (mg/day),

where k_l is the fractional rate of passage of Co.

Two hourly samples of approximately 20 g of duodenal and ileal digesta were collected from Monday up to Thursday in weeks four and six from 08.30 untill 18.30 h. These samples were freeze dried, ground and pooled for each sheep, week and cannula and subsequently analysed for dry matter (DM), ash, Co, Cr and cell wall composition. The daily duodenal or ileal flow of a nutrient was calculated from the ratio of the concentration of that nutrient to the concentration of Co or Cr in duodenal or ileal digesta multiplied by the daily Cr or Co flow. Correction of the ileal flow for withdrawal of digesta from the duodenum was not required, because the concentration of a nutrient relative to the concentration of markers is not affected by duodenal sampling. Flows presented or used for further calculations were averages of estimates based on Co and Cr.

Rumen samples for determination of pH (immediately after sampling) and concentration of volatile fatty acids (VFA), ammonia-N and Co were taken two hourly on Friday in weeks four and six starting at 08.00 h, two hours after the last dosing of Cr-NDF

and Co-EDTA, up to 20.00 h and on Saturday at 08.00 h. Co was also determined in rumen fluid samples taken at 10.00 and 12.00 h on Saturday. Faecal samples (total collection) for analysis of Cr concentration were collected over the following time intervals after the last dosing of Cr-NDF on Friday morning: 24-28, 28-32, 32-36, 36-40, 48-52, 52-56, 56-60, 60-64, 72-76, 76-80, 80-84, 84-88, 96-104, 104-112, 112-120, 120-128 and 128-136 h. The fractional rate of passage of rumen fluid from the rumen (k_l) was estimated from the logarithmic decrease of the Co concentration in rumen fluid and the fractional rate of passage of the particulate phase marker Cr-NDF (k_p) was estimated from the descending part of the logarithmically transferred faecal excretion curve of Cr (Grovum and Williams, 1973).

Collection of faeces and feed residue was done for 10 days starting on Friday 16.00 h (feed) and Saturday 08.00 h (faeces) in weeks three and five and ending on Monday 16.00 h (feed) and Tuesday 08.00 h (faeces) in weeks five and seven. No correction was made for withdrawal of digesta from the duodenum and ileum, which occurred on four of the ten days of faecal collection. Total DM withdrawal during duodenal and ileal sampling was approximately 15 g/day, which averaged over the faecal collection period would mean a maximal underestimation of faecal DM excretion of only 6 g/day.

DM and ash were determined by drying at 103°C and ashing at 550°C, respectively. N was determined by the Kjeldahl method with K_2SO_4 and $CuSO_4$ as catalysts. Ammonia N in rumen fluid was measured by the indophenol method (Scheiner, 1976) using a Hitachi (Tokyo, Japan) U 2000 spectrophotometer at wavelength 634.8 nm after storage with TCA. Co and Cr were determined after wet destruction with an atomic absorption spectrophotometer (Varian (Palo Alto, CA., USA) SpectraA 300; wavelengths 240.7 and 357.9 nm for Co and Cr, respectively). VFA's were determined by gas-liquid chromatography in samples stored with 85 % phosphoric acid (Packard Becker 419 (Chrompack, Bergen op Zoom, Netherlands); 6 ft. glass column of 2 mm i.d. filled with 80-100 mesh Chromosorb 101 (Chrompack, Bergen op Zoom, Netherlands); carrier gas N_2, saturated with formic acid; 190°C; iso-capronic acid as internal standard).

Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to Goering and Van Soest (1970). Hemicellulose was calculated as NDF - ADF and cellulose as ADF - ADL.

Means for each animal within period (average of two repeated measurements) were statistically analysed by the program DBSTAT (Brouwer, 1989) with the model: $Y_{ijkl} = \mu + ration_i + period_j + animal_k + error_{ijkl}$, with total degrees of freedom 16. Differences between ration means were compared by Student's t-test (Snedecor and Cochran, 1967), only if the ration effect was significant.

Results

The N disappearance after 12 hours of rumen incubation in dacron bags was 452, 856 and 447 g/kg for SBP, SBPC and SBPP, respectively (Van Bruchem et al., 1993b). This indicates that protein in SBP and SBPP was partly resistant to degradation in the rumen and that casein had a high rumen degradability. The fraction of the supplementary proteins not degraded in the rumen was highly degradable post ruminally as indicated by the N disappearance from small dacron bags introduced into the proximal duodenum of cattle after pepsin/HCl incubation. N disappearance in the lower gut was 904, 966 and 974 g/kg for SBP, SBPC and SBPP, respectively (Van Bruchem et al., 1993b).

Ammonia treatment increased intake of all constituents of wheat straw (except of lignin) significantly (Tables 4 and 5). The differences between rations with regard to intake of cell wall components as given in Table 5 reflect differences in straw intake, because intake of cell wall components from the supplements were similar for all rations. Intake of NDF, hemicellulose, cellulose and lignin from the supplements were 7.5, 3.4, 3.7 and 0.4 $g/kg^{0.75}$/day, respectively.

Casein and potato protein supplementation had different effects on intake of ammoniated wheat straw. Intake of ammoniated wheat straw was significantly higher for AWSP than for AWSC (Tables 4 and 5). Total OMI of AWSP was significantly higher than that of AWS, as a result of the additional intake of the protein supplement and a higher ($P > 0.05$) intake of ammoniated wheat straw. Total OMI of AWSC and AWS were similar, indicating that OMI of ammoniated wheat straw was substituted by the intake of the additional casein supplement.

For calculation of the digestion of the straw components of whole rations, the dry matter digestion (DMD) and organic matter digestion (OMD) of the supplements were assumed 850 g/kg.

Ammonia treatment significantly increased digestion of DM, OM and cell wall constituents (except lignin) of wheat straw (Tables 4 and 5). As a result of the higher straw intake relative to intake of the supplement for AWS compared with UWS, no significant differences were found for DMD and OMD of these whole rations. Casein supplementation significantly reduced DMD, NDFD and digestion of cellulose of ammoniated wheat straw and for potato protein supplementation to ammoniated wheat straw a reduced ($P < 0.05$) cellulose digestion was observed. DMD, OMD and NDFD of ammoniated wheat straw were significantly lower for AWSC than for AWSP.

Table 4. Intake and digestion of DM and OM, DOMI and average weight of the animals.

	UWS	AWS	AWSC	AWSP	SEM
Intake (g/kg$^{0.75}$/day)					
DM - *whole ration*	45.0^{a}	53.9^{b}	53.9^{b}	61.4^{c}	1.43
- *straw*	26.5^{a}	35.6^{bc}	32.2^{b}	39.0^{c}	1.34
OM - *whole ration*	41.2^{a}	49.2^{b}	49.4^{b}	56.2^{c}	1.32
- *straw*	24.4^{a}	32.6^{bc}	29.4^{b}	35.7^{c}	1.22
Digestion (g/kg)					
DM - *whole ration*	590^{a}	601^{a}	600^{a}	618^{b}	4.1
- *straw*	406^{a}	471^{b}	429^{a}	483^{b}	12.3
OM - *whole ration*	619^{a}	629^{ab}	628^{ab}	643^{b}	4.7
- *straw*	459^{a}	514^{bc}	476^{ab}	523^{c}	12.6
DOMI (g/kg$^{0.75}$/day)					
- *whole ration*	25.5^{a}	30.9^{b}	31.0^{b}	35.9^{c}	0.90
- *straw*	11.2^{a}	16.8^{c}	14.0^{b}	18.5^{c}	0.82
Average weight of the animals (kg)	58.8^{a}	60.4^{ab}	61.3^{ab}	63.0^{b}	1.28

Different superscripts in a row indicate significant differences ($P < 0.05$).

Table 5. Intake (I) and digestion (D) of cell wall components of whole rations.

	UWS	AWS	AWSC	AWSP	SEM
NDF					
I (g/kg$^{0.75}$/day)	28.1^{a}	34.4^{bc}	32.5^{b}	37.6^{c}	1.02
D (g/kg)	597^{a}	647^{c}	622^{b}	642^{c}	6.0
Hemicellulose					
I (g/kg$^{0.75}$/day)	10.9^{a}	12.6^{bc}	12.3^{b}	14.2^{c}	0.43
D (g/kg)	639^{a}	699^{b}	696^{b}	712^{b}	7.3
Cellulose					
I (g/kg$^{0.75}$/day)	15.0^{a}	19.4^{bc}	18.1^{b}	20.8^{c}	0.51
D (g/kg)	659^{a}	699^{b}	666^{a}	674^{a}	7.4
Lignin					
I (g/kg$^{0.75}$/day)	2.2^{a}	2.3^{ab}	2.2^{a}	2.6^{b}	0.11
D (g/kg)	-81^{a}	-93^{a}	-187^{b}	-33^{a}	26.5

Different superscripts in a row indicate significant differences ($P < 0.05$).

Total DOMI (digestible organic matter intake) was significantly higher for AWS than for UWS and significantly higher for AWSP than for AWS and AWSC. Total DOMI was similar for AWS and AWSC. Assuming maintenance requirements of 26 g DOMI/$kg^{0.75}$/day for sheep (adapted from Agricultural Research Council, 1980), the whole rations UWS, AWS, AWSC and AWSP could cover 98, 119, 119 and 138 % of maintenance requirements, respectively.

The ratio Cr/Co introduced into the rumen was on average 1.19. In duodenal samples this ratio was 1.21 (SEM 0.018) and in ileal samples 1.24 (SEM 0.032), indicating that quite representative samples of duodenal and ileal digesta in respect of fluid and particulate phases were obtained.

The partial digestion, defined as the proportion of the apparent whole tract digestion apparently occurring in the rumen, small intestine (SI) or large intestine (LI) is given in Table 6. The partial small intestinal digestion of cell wall components did not differ significantly from zero and was not given in this table.

No differences in contribution of the rumen to whole tract digestion of DM, OM and cell wall constituents were observed between UWS and AWS. Partial rumen digestion was lower for AWSC and AWSP than for AWS (significantly for hemicellulose in the case of AWSC and for OM in the case of AWSP). Rations did not differ significantly with regard to contributions of the small and large intestine to whole tract apparent digestion of DM and OM.

The partial rumen digestion was lower and the partial large intestinal digestion higher for DM than for OM. This could be explained by the flow of ash in the various parts of the digestive tract. Intake, duodenal flow, ileal flow and faecal excretion of ash were averaged across rations (between brackets s.e of mean, corrected for animal, period and ration effects) 100.3 (1.52), 148.0 (2.33), 124.4 (2.76) and 68.8 (3.34) g/day, respectively. This indicates that the large intestine was of more importance for apparent (re-)absorption of ash than the small intestine.

Rumen degradation of cell wall components (g/kg ingested) is given in Table 7. Ammoniation of wheat straw significantly increased rumen degradation of cell wall components. Casein supplementation reduced rumen degradation of cell wall constituents significantly, whereas the reduction in rumen degradation of cell wall components due to potato protein supplementation was only significant for cellulose.

Fermentation parameters are given in Table 8. Rumen fluid samples, in which these parameters were determined were collected two hourly from 08.00 h till 20.00 h. Collection of rumen fluid at 08.00, 12.00, 16.00 and 20.00 h coincided with the moment of feeding, while the rumen fluid collected at 10.00. 14.00 and 18.00 was taken two hours after feeding.

The pH of the rumen fluid was not significantly different between rations, but the pH at feeding was higher than the pH two hours after feeding ($P < 0.05$). The NH_3-N concentration in the rumen fluid was significantly higher for AWSC than for AWS. Ammoniation of wheat straw increased the rumen NH_3-N concentration significantly. The

Table 6. Apparent partial digestion in various sites of the digestive tract (g/kg whole tract apparent digestion).

	UWS	AWS	AWSC	AWSP	SEM
DM: - *rumen*	678	673	598	573	32.2
- *SI*	148	150	208	225	32.6
- *LI*	174	178	194	203	21.2
OM: - *rumen*	775^{b}	773^{b}	703^{ab}	663^{a}	26.2
- *SI*	123	124	175	205	28.0
- *LI*	102	104	123	132	19.0
NDF: - *rumen*	933	935	880	883	23.5
Hemicellulose: - *rumen*	883^{b}	878^{b}	813^{a}	830^{ab}	18.9
Cellulose: - *rumen*	918	930	900	898	26.1

Different superscripts in a row indicate significant differences ($P < 0.05$).

Table 7. Rumen degradation of cell wall components ((intake - duodenal flow)/intake, g/kg).

	UWS	AWS	AWSC	AWSP	SEM
NDF	555^{a}	602^{b}	547^{a}	564^{ab}	12.3
Hemicellulose	564^{a}	614^{b}	565^{a}	592^{ab}	9.6
Cellulose	604^{a}	651^{b}	600^{a}	604^{a}	10.3

Different superscripts in a row indicate significant differences ($P < 0.05$).

NH_3-N concentration in rumen fluid did not differ significantly between samples taken at feeding or two hours after feeding, except for AWSC.

The VFA concentration in the rumen fluid was significantly higher in samples taken two hours after feeding than in samples taken at the moment of feeding. Molar proportions of individual VFA's differed slightly between rations. AWS had a significantly higher proportion of acetate (HAc) and a significantly lower proportion of propionate (HPr) than the other rations. No significant differences emerged between rations in molar proportion of butyrate (HBu). Only very small quantities of iso-valeriate (HiVA), valeriate (HVa) and iso-butyrate (traces, not included in Table 8) were found.

The rumen fluid volume was significantly higher for AWSP than for AWSC (Table 9). Ammoniation of wheat straw significantly increased the rumen fluid volume. No significant differences were observed between rations with regard to k_l and k_p. The k_l and k_p were significantly correlated ($r = 0.854$, $n = 16$, $P < 0.001$). Regression of rumen fluid volume (l) on NDFI (g/day) with inclusion of the animal effect as a factor in the regression model

Table 8. Rumen fermentation parameters

	UWS	AWS	AWSC	AWSP	SEM
pH - *at feeding*	6.35	6.27	6.22	6.26	0.042
- *2 hrs after feeding*	6.22	6.13	6.12	6.10	0.054
- *average*	6.29	6.20	6.17	6.18	0.046
NH3-N (mg/l):					
- *at feeding*	59.1^{a}	154.8^{b}	316.3^{c}	236.1^{bc}	27.24
- *2 hrs after feeding*	50.9^{a}	179.0^{b}	406.7^{c}	266.9^{b}	27.20
- *average*	55.0^{a}	166.9^{b}	361.5^{c}	251.5^{b}	27.12
VFA (mmole/l):					
- *at feeding*	99.6^{a}	105.9^{ab}	112.3^{ab}	115.8^{b}	4.42
- *2 hrs after feeding*	103.2^{a}	113.8^{ab}	123.5^{b}	125.5^{b}	4.01
- *average*	101.4^{a}	109.8^{ab}	117.9^{b}	120.6^{b}	4.10
Molar proportions (mmole/mole):					
HAc	710^{a}	729^{b}	700^{a}	709^{a}	3.2
HPr	188^{b}	177^{a}	194^{b}	188^{b}	3.5
HBu	95	90	89	93	2.9
HiVa	3^{a}	1^{a}	8^{b}	5^{ab}	1.5
HVa	3^{a}	3^{a}	11^{c}	5^{b}	0.6

Different superscripts in a row indicate significant differences ($P < 0.05$).

Table 9. Rumen fluid volume, k_l, k_p and production, rumen pool size, rate of clearance ($k_{cl,VFA}$) and rate of absorption ($k_{abs,VFA}$) of volatile fatty acids.

	UWS	AWS	AWSC	AWSP	SEM
Rumen volume (l)	5.98^{a}	7.47^{bc}	6.63^{ab}	8.89^{c}	0.431
k_l (%/h)	7.5	8.1	8.2	7.4	0.32
k_p (%/h)	3.5	3.8	4.1	3.7	0.17
VFA production	4.7^{a}	5.9^{b}	5.4^{b}	6.0^{b}	0.21
(moles/day)	613^{a}	818^{a}	793^{a}	1065^{b}	68.2
VFA pool (mmoles)	34.5^{a}	31.5^{ab}	31.3^{ab}	23.5^{b}	2.61
$k_{cl,VFA}$ (%/h)	27.2^{a}	23.5^{ab}	23.0^{ab}	16.2^{b}	2.50
$k_{abs,VFA}$ (%/h)					

Different superscripts in a row indicate significant differences ($P < 0.05$).

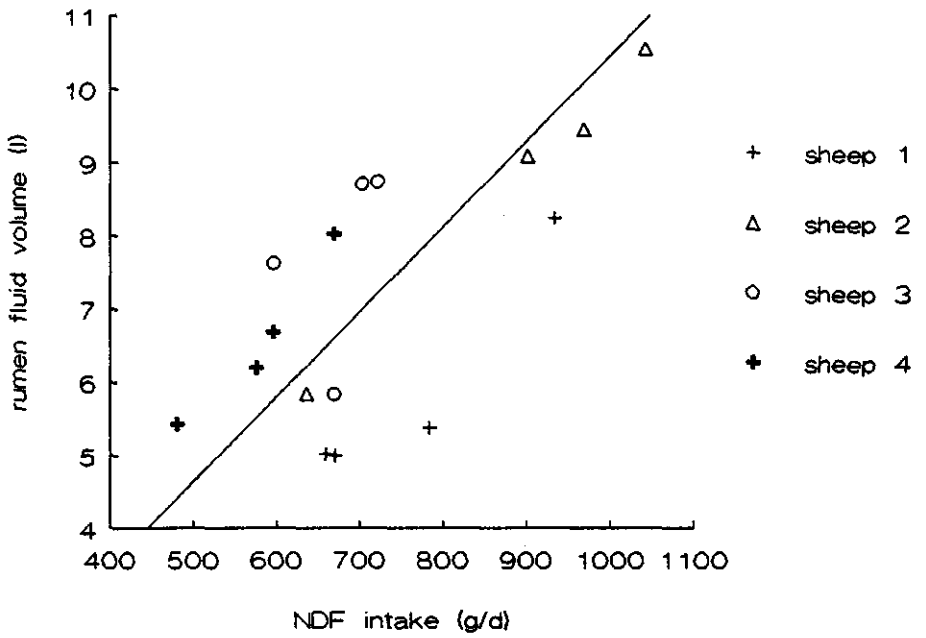

Figure 1. Relationship between rumen fluid volume and NDFI.

yielded the following equation (between brackets s.e. of estimate, intercept average over animals):

Rumen fluid volume = -1.2 (1.29) + 0.012 (0.0017) * NDFI (R^2 = 0.876, n = 16, RSD = 0.73).

The (uncorrected) data and the regression line are plotted in Figure 1.

The rate of clearance of volatile fatty acids ($k_{cl,VFA}$) was calculated as VFA-production/VFA pool. The VFA production was estimated from the apparently rumen degraded organic matter (ARDOM = OMI - OM flow in the duodenum) by assuming a molecular weight of glucose in polymerized carbohydrates of 162 g and a molar VFA production of 100/(0.5*(proportion of HAc + proportion of HPr)+ proportion of HBu) per

mole glucose (Czerkawski, 1986). The rumen VFA pool was estimated as rumen fluid volume * average VFA concentration. The rate of absorption of VFA ($k_{abs,VFA}$) was calculated as $k_{cl,VFA} - k_l$. AWSP tended ($P < 0.10$) to have a lower $k_{cl,VFA}$ and $k_{abs,VFA}$ than AWSC and AWS, but the daily amounts of VFA's absorbed from the rumen were not significantly different between rations. VFA absorption was 3.7, 4.3, 3.9 and 4.2 moles/day (SEM 0.21) for UWS, AWS, AWSC and AWSP, respectively. Absorption of VFA's contributed for 78, 73, 71 and 69 % (SEM 2.0) to the rumen turnover of VFA's for UWS, AWS, AWSC and AWSP, respectively. The $k_{abs,VFA}$ was negatively correlated with rumen VFA pool ($r = -0.850$, $n = 16$, $P < 0.001$). The pH was negatively ($r = -0.462$, $n = 16$, $P = 0.07$) and the concentration of VFA in rumen fluid positively ($r = 0.857$, $n = 16$, $P < 0.001$) correlated with rumen VFA pool.

Discussion

Ad libitum intake of ammoniated wheat straw responded differently to casein than to potato protein supplementation. OMI of ammoniated wheat straw was completely substituted by the casein supplement, whereas potato protein supplementation resulted in a 10 % higher straw OMI. Potato protein was partly degradable in the rumen and casein almost completely. Both protein supplements increased the rumen NH_3-N concentration compared with AWS, casein by 117 % and potato protein by 51 %.

Increased protein availability in the rumen affected cell wall degradation negatively. Rumen degradation of NDF, hemicellulose and cellulose were significantly lower for AWSC than for AWS (9.2, 8.0 and 7.8 % respectively). The reduction in rumen degradation as a result of potato protein supplementation was 6.3, 3.6 and 7.2 % for NDF, which was significant for cellulose.

No significant differences in pH were observed between rations AWS, AWSC and AWSP. The pH was, however, not measured very shortly after ingestion of the protein supplements, when the effect would probably have been largest. It is, however, not likely that the protein supplements would affect pH much, because they comprised only a small proportion (approximately 20 %) of the easily degradable sugar beet pulp supplement. In addition, pH was likely to be buffered by the high ammonia concentration in the rumen fluid of sheep fed AWSC and AWSP.

Accumulation of VFA's may limit microbial activity, even when the pH does not change (Hungate, 1966). The VFA concentration in rumen fluid of sheep fed AWSP and AWSC were slightly, but insignificantly, higher than that in rumen fluid of sheep fed AWS. Whether this rather small difference could account for the difference in rumen degradability is unknown. The relatively high ammonia concentration in the rumen fluid as found for AWSC and AWSP did probably not affect rumen degradability. Satter and Slyter (1974) did not observe toxic effects of ammonia concentrations up to 600 mg/l on microbial protein

synthesis *in vitro* and also in the present experiment no negative effects of casein supplementation on efficiency of microbial protein synthesis was observed (Van Bruchem et al, 1993b).

The retention time of particulate matter in the rumen is equal to $100/k_p$ and was 26.4, 24.3 and 27.0 hours for AWS, AWSC and AWSP, respectively and did not differ significantly between rations either.

Hence, it was unlikely that differences in rumen environment and/or retention time could explain the differences in rumen degradation of cell wall components between rations AWS, AWSC and AWSP.

The lower rumen degradation of cell wall components for protein supplemented ammoniated wheat straw was compensated by a higher large intestinal degradation. Whole-tract digestion of NDF for AWSC and of cellulose for AWSC and AWSP remained, however, significantly lower than of AWS. In turn, the reduction of hemicellulose degradation in the rumen as a result of protein supplementation was completely compensated by an increased hindgut fermentation.

Animals fed AWSP had a higher NDFI than animals fed AWS and AWSC. Thus, if a steady-state situation in the rumen is assumed, the quantity of NDF passing from and/or degraded in the rumen should be more. Daily passage of NDF was 264, 295, 326 and 369 g (SEM 18.4) for UWS, AWS, AWSC and AWSP, respectively, significantly higher for AWSP than for UWS and AWS. The daily quantity of NDF degraded in the rumen was 333, 451, 392 and 473 g (SEM 12.6) for UWS, AWS, AWSC and AWSP, respectively, lowest for UWS ($P < 0.05$) and significantly lower for AWSC than for the other two ammoniated wheat straw-based rations. Thus, the higher NDFI observed for AWSP compared with AWS was associated with a higher passage of NDF from the rumen and the lower NDFI observed for AWSC compared with AWS with a reduced rumen degradation.

Since the k_p of AWS and AWSP did not differ significantly, the higher passage of NDF from the rumen should be attributed to a higher rumen NDF pool for AWSP than for AWS. An indication of the rumen pool size could be obtained from the duodenal NDF flow and k_p. The k_p estimated from faecal excretion of Cr-NDF is not representative for the whole rumen NDF pool, since it refers to small particles only (size of marker in between 0.2 and 1.0 mm) and to particles with a high specific functional gravity and consequently a relatively high k_p (Sutherland, 1987). Hence, k_p estimated by Cr-NDF is overestimating the actual k_p of the small particle rumen NDF pool (Aitchisson et al, 1986). However, rates of passage of various rumen fractions are probably positively correlated, as illustrated by the significant positive correlation between k_p and k_l as observed in the present experiment. Thus, calculation of the rumen small particle NDF pool size from the duodenal NDF flow and k_p probably gives reasonably accurate relative estimates. Estimates of the rumen small particle NDF pool size by the formula: duodenal flow (g)/(k_p (%/h) * 0.24) were 311, 324, 329, and 416 g (SEM 19.0) for UWS, AWS, AWSC and AWSP, respectively, significantly higher for AWSP than for the other rations. Animals fed AWSP would rather have a higher proportion

of large particle NDF in the rumen than a lower compared with animals fed AWS or AWSC, as a result of the higher intake. Therefore, it seems justified to conclude that the total NDF pool was greater for AWSP than for AWS and AWSC. Because there was also no reason to assume that the rumen of animals fed AWSP would contain less non cell wall dry matter than the other rations, it is likely that animals fed AWSP had a higher rumen DM fill. Also the rumen fluid volume was higher for sheep fed AWSP compared with AWS or AWSC. Visual observations supported these findings.

Ammonia treatment of wheat straw increased straw intake and digestion significantly. Ammoniation of wheat straw did not affect partial rumen digestion, rumen pH and rumen VFA concentration significantly. For sheep fed AWS a slightly, but significantly higher molar proportion of acetate was observed. The rate of passage of the fluid and particulate phase was not affected by ammonia treatment. Duodenal passage of NDF was not significantly affected by ammonia treatment and was 264 and 295 g/day for UWS and AWS, respectively. Hence, the increased intake of NDF due to ammonia treatment was associated mainly with an increased rumen degradation of NDF. Animals fed AWS consumed 149 g NDF/day more ($P < 0.05$) than did animals fed UWS, while the rumen degradation of NDF was 118 g more ($P < 0.05$). The rumen small particle NDF pool calculated from the duodenal NDF flow and k_p was 311 g for UWS and 324 g for AWS ($P > 0.05$). Van Bruchem et al. (1993a) observed a significantly lower proportion of small particles in the rumen for AWS than for UWS in sheep. Therefore, the total NDF pool could have been greater for AWS than for UWS in the present experiment. The rumen fluid volume, which is related to the rumen dry matter fill (Owens and Goetsch, 1986) was significantly lower for UWS than for AWS.

The fact that NDFI was highly correlated with rumen fluid volume indicates that other factors than rumen fill were limiting intake of UWS, AWS and AWSC and possibly AWSP. Van Bruchem et al. (1993b) reported a linear relationship between amount of amino acid-nitrogen truly absorbed from the small intestine and DOMI in the present experiment. This suggests that variation in DOMI observed in the present experiment could be explained by variation in small intestinal availability of amino acid-nitrogen in the perspective of a required balance between ketogenic, glucogenic and aminogenic nutrients for metabolism.

The rumen was the most important site for digestion of all nutrients. The relative contribution of the rumen to whole tract digestion of OM and cell wall components did not differ between the present experiment and the earlier experiment reported by Van Bruchem et al. (1993a). However, the results of the present experiment do not support the suggestion by Demeyer (1991) that the importance of hindgut fermentation is increasing with decreasing quality of the ration.

Ruminal apparent OM digestion as a proportion of whole-tract OMD was higher for UWS and AWS (774 g/kg) in the present experiment than the value of 650 g/kg observed

by Zorilla-Rios et al. (1991) for ammoniated and untreated wheat straw fed to cattle.

The rate of absorption of volatile fatty acids did not differ between rations. Although the calculation of the rate of resorption of VFA was based on a number of assumptions, the estimates showed clearly that absorption through the rumen wall is the major way of clearance of VFA's from the rumen. Absorption through the rumen wall contributed on average for 74 % to clearance of VFA's from the rumen. Rates of VFA absorption and contribution of absorption to clearance of VFA's from the rumen were in line with values reported by Tamminga and Van Vuuren (1988) and Dijkstra et al. (1993) for dairy cattle.

Absorption of VFA's from the rumen proceeds through diffusion through the rumen wall and increases with decreasing pH (less dissociated VFA's, the absorption of which is negligible, at low pH) and increasing VFA concentration as a result of a higher concentration gradient between rumen and rumen wall cells (Dijkstra et al., 1993). Despite the fact that pH decreased and VFA concentration increased with increasing rumen VFA pool size in the present experiment, the $k_{abs,VFA}$ decreased with increasing rumen VFA pool size. Dijkstra et al. (1993) reported lower fractional absorption rates of VFA's in dairy cattle with a higher rumen fluid volume. A higher rumen volume increases the average distance of the VFA's in the rumen fluid to the rumen mucosa and hence decreases the diffusion rate. The surface area for absorption increases, however, with increasing rumen fluid volume, which increases the absorption rate. Dijkstra et al. (1993) proposed, therefore, that absorption of VFA's from the rumen should be scaled to the metabolic volume of the rumen ($volume^{0.75}$). In the present experiment, daily absorption of VFA's from the rumen was not significantly different between rations, despite the differences between rations in pH, VFA concentration and rumen fluid volume. It could be, therefore, that the daily absorption of VFA's in the present experiment was limited by an other factor, like blood flow (Dobson, 1984) and/or accumulation of VFA's in rumen wall cells (Dijkstra et al., 1993).

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4.2 Protein supplementation to ammoniated wheat straw. 2. Extent and efficiency of rumen microbial protein synthesis and small intestinal protein disappearance in sheep

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Abstract

The effect of supplementation of casein of a high rumen degradability and potato protein of a relatively low rumen degradability to ammonia-treated wheat straw on extent and efficiency of microbial protein synthesis and small intestinal protein digestion was studied in a 4 x 4 Latin square design with four sheep. Rations offered were: 1) untreated wheat straw (UWS), 2) ammoniated wheat straw (AWS), 3) AWS supplemented with 3.2 g/kg$^{0.75}$/day casein (AWSC) and 4) AWS supplemented with 3.9 g/kg$^{0.75}$/day potato protein (AWSP). Straw was offered *ad libitum* and all rations were supplemented with sugar beet pulp and a mineral mixture. The efficiency of microbial protein synthesis (EMPS) was estimated from the diaminopimelic acid flow in the duodenum (DAPA), from the amino acid profiles of dietary intake, rumen microbes and duodenal digesta (AAP) and from urinary excretion of purine derivatives (PD). The average EMPS across methods of estimation was 23.3, 26.2, 34.8 and 31.7 g N/kg apparently rumen degraded organic matter for UWS, AWS, AWSC and AWSP, respectively. The average EMPS and also the estimates based on DAPA and AAP were not significantly different between UWS and AWS, but EMPS based on PD was significantly higher for AWS than for UWS. All methods of estimation showed, that protein supplementation to AWS significantly increased EMPS. Only the estimate of EMPS based on AAP was significantly higher for AWSC than for AWSP. The rumen degradation of feed amino acid nitrogen (AA-N) was significantly higher for AWSC than for the other rations. The apparent small intestinal digestion of AA-N and N was significantly higher for AWSP than for the other rations. The true small intestinal digestion was 0.86, 0.84 and 0.68 for AA-N, N and non protein nitrogen, respectively. Ileal endogenous losses of AA-N approximated 6 mg/g duodenal non protein dry matter flow. Linear relationships between N balance and digestible organic matter intake (DOMI) and truly absorbed AA-N and DOMI were observed. Regression of N balance on truly absorbed AA-N resulted in an estimate of net efficiency of utilization of truly absorbed AA-N of 0.54.

Introduction

In ruminants, roughage intake may be limited by the intestinal supply of amino acids (Egan and Moir, 1965; Egan, 1977; Egan and Doyle, 1985; Preston and Leng, 1987; Doyle and McLaren, 1988; Doyle and Panday, 1990). The availability of amino acids for absorption from the small intestine depends on the amount of amino acids entering the duodenum, either from feed or from microbial origin and on the quantity of endogenous amino acids passing from the ileum to the caecum/colon (Van Bruchem et al., 1989). Limited rumen availability of nutrients as peptides, branched chain volatile fattty acids and/or minerals in combination with a long retention time of microbes in the rumen may be reasons for the relatively low efficiency of microbial protein synthesis (Hespell and Bryant, 1979) as found for untreated and ammoniated wheat straw (Oosting et al., 1993a). Endogenous protein losses from the small intestine were related to the non protein dry matter passage (NPDM) in the duodenum by Van Bruchem et al. (1989). The endogenous amino acid losses per kg NPDM flow in the duodenum were lower for rations based on untreated and ammoniated wheat straw (Oosting et al., 1993a) than for rations based on forages grown under temperate conditions (Van Bruchem et al., 1989). Hence, major constraints to amino acid availability in the small intestine for rations with straw as the major component are the low efficiency of microbial protein synthesis in the rumen in combination with a low flow of amino acids from straw origin with probably a low small intestinal digestibility (Hvelplund, 1989).

The present experiment was conducted to study, for ammoniated wheat straw, the effects of protein supplements varying in rumen degradability on efficiency of microbial protein synthesis and small intestinal disappearance of amino acids. Results with regard to intake and site and extent of digestion were reported elsewhere (Oosting et al., 1993b).

Materials and methods

The experimental procedure was as given by Oosting et al. (1993b). A summary is given hereunder. Materials and methods not described by Oosting et al. (1993b) are given in full detail.

Four sheep of approximately 61 kg were fitted with a small cannula in the dorsal rumen sac and with T-shape cannulas in the proximal duodenum and terminal ileum. The animals were housed in metabolism cages and received equal portions of their rations every four hours starting at 00.00 h. Four rations were offered in a 4x4 Latin square design with experimental periods of seven weeks, of which the first three for adaptation: 1) untreated wheat straw (UWS), 2) ammoniated wheat straw (AWS), 3) AWS supplemented with casein (3.2 g/kg$^{0.75}$/day) and 4) AWS supplemented with potato protein (3.9 g/kg$^{0.75}$/day). Straw was offered *ad libitum* (at least 25 % excess). All rations were supplemented with sugar beet pulp (16.7 g DM/kg$^{0.75}$/day) and a mineral/vitamin mixture (1.8 g DM/kg$^{0.75}$/day). Sugar beet

pulp, minerals, vitamines and the protein supplements were mixed and offered as a pellet.

Estimation of flows of nutrients in the duodenum and ileum were based on introduction into the rumen of 10 g Cr-NDF and 3 g Co-EDTA, both prepared according to Udén et al. (1980), per day at intervals of 6 h, thus achieving an average hourly flow of 22.0 mg Cr and 18.5 mg Co. Samples of about 20 g of duodenal and ileal digesta were collected every two hours starting at 08.30 till 18.30 h during four consecutive days in the fourth and sixth week of each experimental period. These samples were freeze dried, ground through a 1 mm sieve and pooled for each sheep, cannula and week and subsequently analysed for dry matter (DM), ash, Co, Cr, nitrogen (N) and amino acids.

During the four days in the fourth and sixth week of each experimental period, when samples of duodenal and ileal digesta were taken, rumen fluid samples of 50 ml were taken just after feeding at 08.00 h and in between two feedings at 10.00 h. From these samples, rumen microbes were isolated by differential centrifugation (550 g in an Omnifuge 2.0 RS and 70,000 g in an MSE superspeed 65 centrifuge) at 4°C. The pellet was washed twice with a buffer prepared according to Meyer et al. (1967). After freeze drying, grinding through a 1 mm sieve and pooling for each animal and week, samples were properly stored pending N and amino acid analyses. At the same days in weeks four and six of each experimental period, thus, while a steady state concentration of Co was present in the rumen, rumen fluid samples of 50 ml were taken at 08.00 and 10.00 h, two and four hours after introduction of the marker into the rumen, respectively, for analysis of Co and diaminopimelic acid (DAPA). The procedure for estimation of the rumen fluid volume from the average Co concentration in these samples was given by Oosting et al. (1993b). DM and DAPA were measured in these rumen fluid samples pooled for each animal and week.

Collection of feed residues, faeces and urine was done for ten days twice in each experimental period, once from week three to five and once from week five to seven. Faecal excretion was not corrected for duodenal and ileal sampling, which was done on four of the ten days of faecal collection.

For the feeds in the present experiment, the disappearance of N from small dacron bags introduced into the duodenal cannula was measured in cattle. Untreated wheat straw and ammoniated wheat straw and the supplements sugar beet pulp (SBP), sugar beet pulp with caseine (SPBC) and sugar beet pulp with potato protein (SBPP), all ground through a 1 mm sieve were incubated in dacron bags (size 70 x 120 mm, pore size 41 μm x 41 μm) in triplicate (straw) or sixfold (supplements) in the rumen of two cows for 12 h. Straw was also incubated for 24 and 48 h. After collection of the bags and washing, the residue was dried at 70°C for 48 h and pooled for each feed. A sub-sample was taken for DM and N analysis and 18 sub-samples of approximately 0.5 g of each feed were weighed into small dacron bags (size: 3 x 6 cm, poresize 41 μm x 41 μm). At the Institute for Livestock Feeding and Nutrition Research (IVVO-Lelystad, The Netherlands) these samples were incubated in pepsin-HCl (1 g pepsin/l, 1 N HCl) at 39°C for 1 h and subsequently introduced into the distal duodenum of 6 cows in triplicate per feed per cow. After collection of the bags voided with the faeces, washing in tap water and drying at 70°C for 48 h, the residues were pooled

for each feed and analysed for DM and N.

The duodenal flows of microbes were estimated by the following methods:

1) DAPA method, based on the daily DAPA flow in the duodenum and the DAPA/N ratio in isolated rumen bacteria,

2) AAP-method, based on the amino acid profiles (AAP) of ingested protein, bovine pepsinogen (Siddons et al., 1982), microbial protein and duodenal digesta. Dietary, pepsinogen and microbial amino acids were mixed by an iterative procedure in such proportions, that the computed AAP matched best to the actual AAP of duodenal protein. This was tested by minimizing the objective function:

$$\sum_{AA=1}^{AA=16} (1 - AA_{computed}/AA_{actual})^2.$$

This procedure was done for 16 amino acids, leaving out cystine due to the high analytical variation and tryptophan, because the tryptophan concentration in pepsinogen was unknown.

3) PD method, based on excretion of purine derivatives in the urine measured according to the method described by Chen et al. (1990b). From the daily urinary excretion of purine derivatives the corresponding amount of microbial purines absorbed by the animal was estimated based on the model described by Chen et al. (1990a). The duodenal flow of microbial N was then calculated from the absorbed quantity of microbial purines by accepting a digestibility of microbial purines of 0.83 and a purine-N/total microbial-N ratio of 0.116 (Chen et al., 1991).

The true digestion of individual and total amino acids (TAA), amino acid-N (AA-N), N and non protein N (NPN) in the small intestine or over the whole digestive tract (only for N) was estimated by Lucas equations (Van Soest, 1982) of the following general form:

$$DX = a + b*X,$$

where X and DX represent the concentrations of a nutrient and the disappeared nutrient, respectively (in the duodenal flow of non protein dry matter (NPDM) for estimation of the small intestinal true digestion and in OMI for estimation of the whole-tract true digestion), *a* is the endogenous loss of the nutrient per 100 g duodenal NPDM flow or OMI and *b* is the true digestion as fraction. Scaling of DX and X to duodenal NPDM flow for estimation of true small intestinal digestion was proposed by Van Bruchem et al. (1989).

DM was determined by drying at 103°C and ashing was done at 550 °C. N was determined by the Kjeldahl method with K_2SO_4 and $CuSO_4$ as catalysts. Amino acids including DAPA were determined as described by Van Bruchem et al. (1988) with a Biotronic LC5001 automatic amino acid analyzer. Samples were hydrolysed under reflux with

HCl (6 N) at 110 °C for 22 h. The sulphur containing amino acids were determined as methionine sulphone and cysteic acid, respectively, after performic acid oxidation (16 h at 4°C) and hydrolization with 6 N HCl at 110°C for 22 h (Moore, 1963). Tryptophan was analysed after alkaline hydrolysis. A sample containing 25-50 mg protein was mixed in a test tube with 16 ml 4 N LiOH. After cooling in ice, the tubes were made vacuum and subsequently put into an oven at 120°C. After boiling for 16 h the pH was brought down to 4.5 by adding HCl (12 N) under continuous stirring. Then the sample was centrifugated at 550 g and the supernatant quantitatively transferred into a 50 ml volumetric flask and filled to the mark. Five ml of this solution were mixed with a phosphate buffer (11.4 g $K_2HPO_4.3H_2O$ and 6.8 g KH_2PO_4 per liter H_2O) and an internal standard (methyl-tryptophan, 1 mmol/l). After centrifugation with an MSE superspeed 65 centrifuge at 70,000 g, the supernatant was analysed by HPLC (isocratic system, eluens: 13.6 g Na-acetate.$3H_2O$ and 5.7 ml acetic acid in two liters H_2O to which 353 ml methanol were added; 10 cm Lichiosorb RP-18 column; UV detection at 280 nm).

Co and Cr were determined after wet destruction with an atomic absorption spectrophotometer (Varian (Palo Alto, CA., USA) SpectraA 300, at wavelengths of 240.7 and 357.9 nm, respectively).

Means for each animal within period (means of two repeated measurements) were statistically analysed by the program DBSTAT (Brouwer, 1989) with the model:

$$Y_{ijkl} = \mu + ration_i + period_j + animal_k + error_{ijkl},$$

with total degrees of freedom 16. Significance of differences between ration means were compared by two tailed Student's t-test in case the ration effect was significant (Snedecor and Cochran, 1967).

Results

Table 1 presents the composition of the ration constituents untreated and ammoniated wheat straw, sugar beet pulp (SBP), sugar beet pulp + casein (SBPC) and sugar beet pulp + potato protein (SBPP). Ammonia treatment affected neither the amino acid (AA) concentration nor the AAP of wheat straw. The AAP of the supplementary casein and potato protein differed. SBPC had lower molar proportions of aspartic acid, glycine and alanine and higher molar proportions of glutamic acid and proline than SBPP.

Table 1. Composition of diet constituents.

	Untreated wheat straw	Ammoniated wheat straw	SBP	SBPC	SBPP
DM (g/kg)	910	905	848	816	840
Amino acids (mol/kg DM)	0.192	0.189	0.591	1.879	1.606
AAP (mol/kmol):					
cystine	10.4	11.3	11.5	4.7	8.9
aspartic acid	104.9	102.1	86.2	70.9	115.6
methionine	14.9	13.3	15.5	23.6	18.4
threonine	58.3	57.1	59.4	49.0	58.1
serine	74.3	72.1	76.9	73.6	68.9
glutamic acid	122.1	121.6	110.9	162.6	102.7
proline	57.4	60.6	59.9	104.0	54.1
glycine	103.8	108.0	89.3	46.5	80.6
alanine	96.9	97.6	80.2	52.3	69.8
valine	73.1	71.5	83.0	75.9	78.0
isoleucine	37.2	37.7	45.6	51.2	52.0
leucine	58.7	58.8	70.1	85.3	85.2
tyrosine	15.4	15.3	35.8	34.1	34.5
phenylalanine	31.8	30.7	28.6	36.8	41.3
lysine	53.4	58.4	46.1	30.2	27.7
histidine	42.3	39.8	56.5	63.2	59.7
arginine	36.0	33.1	37.0	28.9	35.1
tryptophan	9.1	11.0	7.6	7.2	9.4
N (g/kg DM)	5.8	13.5	14.6	39.3	37.2
AA-N (g/kg DM)	3.4	3.4	10.5	32.0	27.6
AA-N/N (g/kg)	591	250	721	814	744

Table 2 shows the disappearance of N from dacron bags incubated in the rumen and subsequently introduced into the duodenum after pepsin/HCl incubation. Apparent N disappearance in the rumen decreased with incubation time for untreated wheat straw, which should be attributed to contamination with microbial N. For untreated wheat straw DM disappearance from dacron bags incubated in the rumen was 227, 318 and 488 g/kg for incubation periods of 12, 24 and 48 h, respectively. Also for ammoniated wheat straw contamination with microbial N was likely, since N disappearance from dacron bags incubated in the rumen did not change with incubation period, while DM disappearance was 190, 397 and 615 g/kg after rumen incubation for 12, 24 and 48 h, respectively. For both straws, post ruminal N disappearance from dacron bags (pepsin/HCl incubation of residue after rumen incubation followed by introduction into the duodenum) and whole tract N disappearance increased with increasing duration of pre-incubation in the rumen.

Ruminal N disappearance from dacron bags was higher for SBPC than for SBP and SBPP. Ruminal DM disappearance was 638, 772 and 599 g/kg for SBP, SBPC and SBPP, respectively. Post ruminal and whole tract N disappearance of all supplements was high,

though higher for SBPC and SBPP than for SBP.

The AAP's of ingested protein, endogenous protein (bovine pepsinogen), rumen microbes and duodenal digesta are given in Table 3. The microbial AAP did not differ significantly between rations and also no significant differences were observed between UWS and AWS with regard to AAP of dietary intake and duodenal digesta. For presentation in Table 3 these AAP's were combined. The computed proportions of microbial AA as percentage of the duodenal AA flow were significantly higher for AWSC and significantly lower for AWSP than for UWS and AWS. The proportion of endogenous AA in the duodenal protein flow did not differ between rations.

Table 2. Disappearance of N from dacron bags (g/kg).

	Rumen	Intestines[1]	Whole-tract[2]
Untreated wheat straw:			
- *12 h*[3]	221	652	729
- *24 h*	147	715	756
- *48 h*	86	774	794
Ammoniated wheat straw:			
- *12 h*	531	607	815
- *24 h*	549	672	852
- *48 h*	522	750	880
SBP			
- *12 h*	452	904	947
SBPC			
- *12 h*	856	966	995
SBPC			
- *12 h*	447	974	985

1: incubation of residue after rumen incubation in pepsin/HCl followed by introduction into the duodenum and collection in faeces)
2: N disappearance from rumen and intestines
3: duration of rumen incubation

Table 3. AAP (mol/kmol) of dietary intake, bovine pepsinogen, rumen microbes and duodenal digesta.

	AAP dietary intake				AAP endogenous[1]	AAP rumen microbes	AAP duodenal digesta			
	UWS/ AWS	AWSC	AWSP	SEM			UWS/ AWS	AWSC	AWSP	SEM
cys	11.2^{c}	5.5^{a}	9.2^{b}	0.40	n.a.	7.6	12.6^{b}	12.7^{b}	10.9^{a}	0.29
asp	92.2^{b}	75.0^{a}	113.4^{c}	0.29	115.0	114.3	106.7^{a}	105.6^{a}	109.4^{b}	0.72
met	14.9^{a}	22.3^{c}	17.5^{b}	0.35	10.8	19.4	15.9^{a}	17.1^{b}	16.8^{b}	0.23
thr	58.8^{b}	50.1^{a}	57.9^{b}	0.23	76.3	62.6	61.7^{b}	61.1^{a}	61.7^{b}	0.17
ser	75.6^{c}	73.5^{b}	69.5^{a}	0.35	143.2	59.1	63.8^{b}	62.6^{a}	65.1^{c}	0.32
glu	114.8^{b}	157.2^{c}	105.9^{a}	0.66	92.2	104.2	106.3^{b}	107.9^{b}	100.1^{a}	1.07
pro	59.7^{b}	98.4^{c}	55.4^{a}	1.21	44.3	34.9	43.6^{a}	46.1^{b}	48.5^{c}	0.49
gly	94.2^{c}	54.6^{a}	88.3^{b}	0.46	99.4	92.4	93.3^{b}	92.1^{b}	90.3^{a}	0.35
ala	86.2^{c}	58.2^{a}	74.5^{b}	0.61	46.0	103.1	96.8^{b}	96.1^{b}	87.4^{a}	0.52
val	79.3^{b}	76.5^{a}	76.9^{a}	0.55	71.2	71.6	71.7	71.8	73.5	0.55
ile	42.7^{a}	49.4^{b}	49.6^{b}	0.17	90.1	57.5	55.0^{a}	55.2^{a}	58.6^{b}	0.35
leu	66.2^{a}	81.8^{c}	80.6^{b}	0.32	72.0	70.6	73.4^{a}	73.6^{a}	80.5^{b}	0.64
tyr	28.7^{a}	31.6^{b}	31.2^{b}	0.23	50.8	33.8	28.6^{a}	28.6^{a}	30.5^{b}	0.20
phe	29.4^{a}	36.0^{b}	39.5^{c}	0.81	42.9	37.6	37.1^{a}	37.1^{a}	40.6^{b}	0.38
his	49.5^{b}	33.9^{a}	32.9^{a}	1.10	5.6	26.5	31.5^{b}	31.5^{b}	29.4^{a}	0.52
lys	51.2^{a}	60.1^{c}	56.3^{b}	0.32	23.0	65.5	63.3^{b}	63.0^{b}	61.9^{a}	0.26
arg	36.1^{b}	29.4^{a}	34.7^{b}	0.40	17.7	31.3	30.9	30.1	31.2	0.43
try	8.5^{b}	7.7^{a}	9.7^{c}	0.09	n.a.	8.1	8.2	8.1	8.2	0.12
Microbial AA[2]							69.6^{b}	82.1^{c}	53.0^{a}	1.80
Feed AA[2]							28.1^{b}	16.9^{a}	44.6^{c}	1.68
Endogenous AA[2]							2.2	1.1	2.2	0.91

n.a.: not available
1: Composition of bovine pepsinogen adapted from Siddons et al. (1982).
2: Percentage of duodenal AA flow.
Different superscripts in a row indicate significant differences ($P < 0.05$).

The composition of rumen microbes, the rumen microbial N pool calculated from the rumen fluid pool size and the DAPA concentration in rumen fluid and the efficiency of microbial protein synthesis (EMPS) are given in Table 4. No significant ration effects were observed for DAPA, AA, N or AA-N concentrations in microbial DM. A significant ration effect was found for the ratio's AA-N/N and DAPA-N/N, but differences were only small. AWSP had a significantly higher microbial N pool associated with rumen fluid than the other rations, which was mainly caused by differences in the rumen fluid pool size. The DAPA concentration in the rumen fluid did not differ significantly between rations. Over rations the average DAPA concentration in rumen fluid was 0.113 mmol/l (SEM 0.0152), which corresponded to an average concentration of microbial N in rumen fluid of 470 mg/l (SEM 10.7).

Ammoniation of wheat straw did not affect EMPS based on AAP or DAPA significantly, but the EMPS derived from the PD method was significantly higher for AWS than for UWS. Protein supplementation to AWS increased EMPS, although the difference between AWS and AWSP was not significant for EMPS estimated by the PD method. Estimates of EMPS for AWSC and AWSP only differed significantly for those based on AAP. Averages over the three methods of estimation of EMPS were 23.3, 26.2, 34.8 and 31.7 g N/kg (SEM 1.63) apparently rumen degradable organic matter intake (ARDOMI) for UWS, AWS, AWSC and AWSP, respectively. These values were significantly higher for AWSC and AWSP than for UWS and AWS.

The difference between estimates of EMPS based on PD and AAP or DAPA were significantly higher than zero for AWS, indicating, that PD gave significantly higher estimates than the other methods. For AWSC the EMPS based on DAPA was lower than those based on PD ($P > 0.05$) and AAP ($P < 0.05$). For UWS and AWSP no significant differences between methods were observed. EMPS estimates derived from the various methods were significantly correlated. The correlation coefficient between DAPA and AAP was 0.84 ($n = 16$, $P < 0.001$), between DAPA and PD 0.62 ($n = 16$, $P < 0.01$) and between AAP and PD 0.63 ($n = 16$, $P < 0.01$).

Table 4. Composition of rumen microbes, rumen microbial pool associated with fluid and efficiency of microbial protein synthesis (EMPS).

	UWS	AWS	AWSC	AWSP	SEM
DAPA (mmol/kg DM)	17.2	16.5	16.5	17.4	0.49
AA (mol/kg DM)	2.87	2.68	2.81	2.99	0.072
N (g/kg DM)	70.2	66.7	71.0	73.6	1.57
AA-N (g/kg DM)	48.1	44.9	47.1	50.1	1.22
AA-N/N (g/kg)	685^{b}	675^{ab}	663^{a}	683^{b}	4.0
DAPA-N/N (g/kg)	6.88^{b}	6.88^{b}	6.50^{a}	6.59^{ab}	0.095
Microbial N pool associated with fluid (g)	2.72^{a}	3.44^{ab}	3.02^{a}	4.54^{b}	0.373
EMPS (g N/kg ARDOMI):					
- *AAP*	23.8^{a}	24.5^{a}	37.0^{c}	30.4^{b}	1.70
- *DAPA*	22.7^{a}	23.4^{a}	31.6^{b}	31.7^{b}	2.35
- *Purine derivates*	23.3^{a}	30.6^{b}	35.8^{c}	33.2^{bc}	1.08

Different superscripts in a row indicate significant differences ($P < 0.05$).

Table 5 gives the flows of OM, N and AA-N at the various sites of the digestive tract. Ammoniation of wheat straw increased OMI, duodenal and ileal OM flow and faecal excretion of OM significantly. AWSP had significantly higher OMI, duodenal OM flow and faecal OM excretion than AWS, while AWSC and AWS did not differ with regard to OM flows at the various sites of the digestive tract.

The difference in N intake (NI) between UWS and AWS was 7.2 g/day of which 85 % was as a result of N added through ammonia treatment. NI and AA-NI were significantly higher for AWSC and AWSP than for AWS, without significant differences between these rations.

The difference between OMI and duodenal OM flow is equal to intake of apparently degradable organic matter (ARDOMI). Estimates of ARDOMI were 421, 517, 480 and 532 (SEM 18.6) g/day for UWS, AWS, AWSC and AWSP, respectively. ARDOMI was significantly lower for UWS than for AWS and AWSP. The duodenal N and AA-N flows were higher than the intake of these nutrients for all rations, except for AWSC. Applying the proportions of microbial, feed and endogenous AA in the duodenal digesta derived from the AAP method to the duodenal AA-N flow resulted in significantly higher microbial flows for AWSC and AWSP than for AWS and UWS. AWSP had a significantly higher AA-N flow from feed origin and from endogenous origin than the other rations. The AA-N/N ratio in duodenal digesta was significantly higher for UWS than for AWS and was significantly higher for AWSP than for AWS and AWSC. Estimates of the rumen degradation of AA-N from feed origin were 499, 524, 862 and 444 g/kg for UWS, AWS, AWSC and AWSP, respectively (SEM 20.4), when calculated from intake and duodenal flow of feed AA-N.

The AA-N/N ratio in ileal digesta was significantly higher for UWS than for the other rations. Some disappearance of N occurred between the terminal ileum and faeces. The average N disappearance in the large intestine was 183 g/kg ileal N flow (SEM 20.6), without any significant ration effect.

Regression of DNI/OMI on NI/OMI gave estimates for metabolic faecal N excretion of 0.72 (s.e. 0.059) g/100 g OMI and true whole tract N digestion of 0.92 (s.e. 0.032). The residuals (DNI/OMI predicted minus DNI/OMI observed) were not significantly different from zero for any ration.

Urinary N excretion and N balances differed significantly between rations.

Table 5. Intake and flow at the duodenum and ileum and faecal and urinary excretion.

	UWS	AWS	AWSC	AWSP	SEM
Intake (g/day):					
- OM - *total*	872^{a}	1070^{b}	1090^{b}	1260^{c}	33.5
- *straw*	516^{a}	709^{bc}	649^{b}	800^{c}	30.5
- N - *total*	9.0^{a}	16.2^{b}	28.2^{c}	30.4^{c}	0.86
- *straw*	3.3^{a}	10.4^{bc}	9.6^{b}	11.8^{c}	0.56
- AA-N - *total*	6.0^{a}	6.7^{a}	17.6^{b}	16.8^{b}	0.68
- *straw*	2.0^{a}	2.7^{bc}	2.5^{b}	3.1^{c}	0.15
Duodenal flow (g/day):					
- OM	451^{a}	552^{b}	611^{b}	728^{c}	29.1
- N	15.5^{a}	21.3^{b}	26.0^{c}	34.7^{d}	1.23
- AA-N	10.0^{a}	11.8^{ab}	14.3^{b}	20.8^{c}	0.83
- *microbial*	6.8^{a}	8.4^{a}	11.7^{b}	11.0^{b}	0.50
- *feed*	3.0^{a}	3.2^{a}	2.4^{a}	9.4^{b}	0.43
- *endogenous*	0.2^{a}	0.2^{a}	0.2^{a}	0.4^{b}	0.02
- AA-N/N	647^{c}	560^{a}	551^{a}	599^{c}	5.0
Ileal flow (g/day):					
- OM	385^{a}	470^{b}	494^{bc}	561^{c}	24.4
- N	7.7^{a}	11.5^{b}	13.1^{bc}	15.1^{c}	0.66
- AA-N	4.3^{a}	5.1^{ab}	5.6^{b}	6.9^{c}	0.38
- AA-N/N	553^{b}	446^{a}	432^{a}	454^{a}	6.7
Faecal excretion (g/day):					
- OM	331^{a}	397^{b}	407^{b}	454^{c}	13.8
- N	6.7^{a}	9.7^{b}	10.4^{bc}	11.5^{c}	0.372
Urinary N excretion (g/day)	3.6^{a}	7.1^{b}	17.4^{d}	15.9^{c}	0.300
N balance (mg/kg$^{0.75}$/day)	-61.0^{a}	-24.9^{b}	10.2^{c}	131.0^{d}	5.18

Different superscripts in a row indicate significant differences ($P < 0.05$).

The apparent small intestinal digestibilities of AA-N and N were significantly higher for AWSP than for the other rations, while no significant ration effect was found for apparent small intestinal digestibility of NPN (Table 6). Ration differences as found for apparent small intestinal digestion of N and AA-N were not found for true small intestinal digestion (Table 7), indicating that ration differences with regard to apparent small intestinal digestion were related to different duodenal non protein dry matter (NPDM) and duodenal AA-N or N flows. The regression of apparent small intestinally disappeared individual amino acids, TAA, AA-N, N or NPN on duodenal flow of these nutrients (Y and X scaled to NPDM) included all 32 observations. By inclusion of ration as a factor in the regression model it was tested, whether there were significant differences between rations with regard to endogenous ileal losses.

The NPDM flows were 463, 560, 586 and 659 (SEM 26.3) g/day for UWS, AWS, AWSC and AWSP, respectively. Duodenal NPDM flows were significantly higher for AWS and AWSC than for UWS and significantly lower for AWS than for AWSP. For AA-N, TAA and individual amino acids no significant deviations from the general regression were observed as illustrated in Figure 1 for AA-N, while for N and NPN a significant ration effect was found as illustrated in Figures 2 and 3. No significant differences were observed between true small intestinal digestibility of N and AA-N, although NPN had a lower true digestibility than AA-N. Cystine, histidine and tryptophan had true digestibilities lower than 0.80 (Table 7). In addition, molar proportions in ileal endogenous protein losses of cystine and histidine were higher than those in duodenal protein resulting in even lower apparent small intestinal digestibilities for these amino acids. The AAP of endogenous ileal protein losses differed considerably from that of lean lamb meat (Lawrie, 1979), especially for the essential amino acids arginine, lysine and histidine.

Table 6. Apparent small intestinal digestibility (g/kg).

	UWS	AWS	AWSC	AWSP	SEM
N	501[a]	462[a]	494[a]	566[b]	18.1
AA-N	573[a]	571[a]	604[a]	671[b]	13.0
NPN	366	322	359	408	28.6

Different superscripts in a row indicate significant differences ($P < 0.05$).

Table 7. True small intestinal digestibility (TD) and endogenous ileal losses.

	TD (s.e.)	Endogenous ileal loss (s.e.)[1]	AAP of ileal endogenous protein loss (mol/100 mol)[2]
cys	0.67 (0.073)	0.64 (0.130)	1.9 (0.7)
asp	0.84 (0.016)	3.25 (0.267)	9.5 (8.5)
met	0.90 (0.032)	0.41 (0.079)	1.2 (2.0)
thr	0.84 (0.022)	2.29 (0.205)	6.7 (5.4)
ser	0.82 (0.022)	2.50 (0.207)	7.3 (4.9)
glu	0.86 (0.025)	3.74 (0.380)	10.9 (13.0)
pro	0.85 (0.020)	1.87 (0.133)	5.4 (5.5)
gly	0.81 (0.021)	3.53 (0.293)	10.3 (11.8)
ala	0.84 (0.026)	3.37 (0.356)	9.8 (9.4)
val	0.88 (0.018)	2.70 (0.189)	7.9 (5.7)
ile	0.89 (0.015)	1.54 (0.120)	4.5 (4.8)
leu	0.90 (0.018)	2.32 (0.199)	6.7 (7.5)
tyr	0.88 (0.022)	0.79 (0.097)	2.3 (2.3)
phe	0.88 (0.023)	1.12 (0.129)	3.3 (3.1)
his	0.78 (0.049)	1.88 (0.223)	5.5 (2.3)
lys	0.86 (0.014)	1.33 (0.133)	3.9 (6.9)
arg	0.91 (0.018)	0.86 (0.083)	2.5 (5.2)
try	0.76 (0.047)	0.24 (0.057)	0.7 (0.8)
TAA	0.86 (0.018)	35.0 (2.61)	
AA-N	0.86 (0.017)	0.60 (0.044)	
N	0.84 (0.032)	1.11 (0.069) UWS 1.45 (0.083) AWS/AWSC/AWSP	
NPN	0.68 (0.074)	0.34 (0.073) UWS 0.58 (0.110) AWS/AWSC/AWSP	

1: mmol/100 g NPDM for individual amino acids and TAA, g/100 g NPDM for AA-N, N and NPN.
2: between brackets AAP (mol/100 mol) of lean lamb meat (Lawrie, 1979).

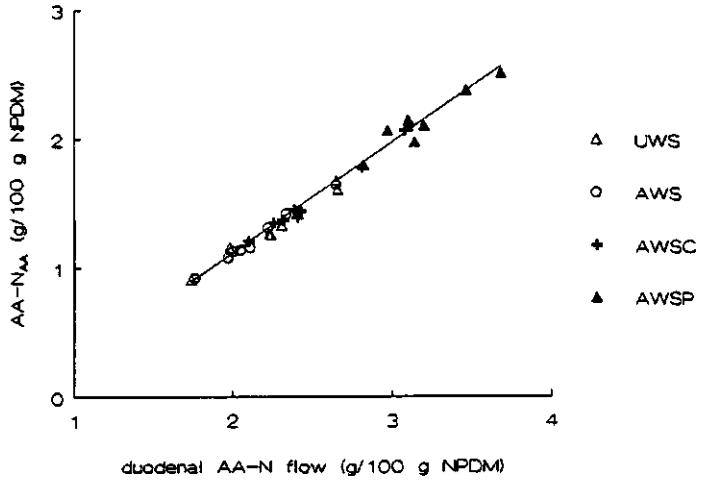

Figure 1. True small intestinal digestion of AA-N. Relationship between apparently small intestinally absorbed AA-N (AA-N_{AA}) and duodenal AA-N flow, both scaled to NPDM.

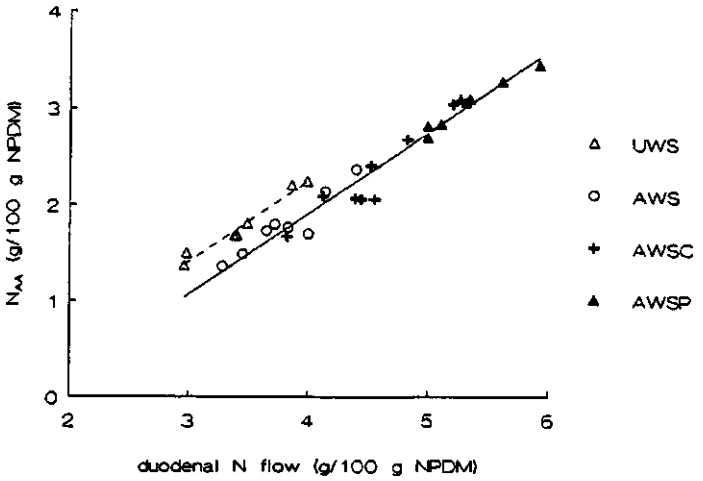

Figure 2. True small intestinal digestion of N. Relationship between apparently small intestinally absorbed N (N_{AA}) and duodenal N flow, both scaled to NPDM.

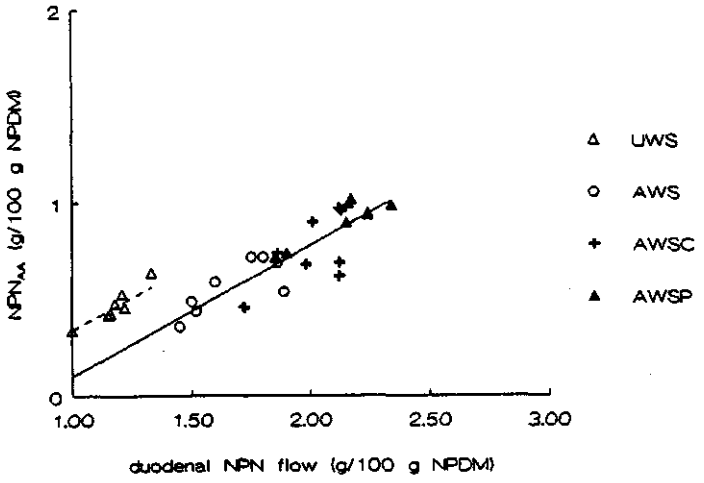

Figure 3. True small intestinal digestion of NPN. Relationship between apparently small intestinally absorbed NPN (NPN_{AA}) and duodenal NPN flow, both scaled to NPDM.

Discussion

Intake and protein status

Intake of roughages could be limited by the amount of protein available for absorption from the small intestine as suggested by Egan (1965), Egan and Moir (1965), Egan (1977), Egan and Doyle (1985), Preston and Leng (1987), Doyle and McLaren (1988) and Doyle and Panday (1990), although no effects of increased small intestinal protein availability were found in several other experiments (Kellaway and Leibholz, 1983; Gherardi and Black, 1989; Ketelaars and Tolkamp, 1991).

In the present experiment DOMI was linearly related to availability of truly absorbed AA-N (AA-N_{TA} = duodenal AA-N flow * 0.86). Regression of AA-N_{TA} on DOMI with inclusion of the period effect as a factor yielded the following relationship (intercept: mean over periods, between brackets s.e. of estimate):

AA-N_{TA} (mg/kg$^{0.75}$/day) = -459 (163.9) + 33.0 (5.27) * DOMI (g/kg$^{0.75}$/day), (R^2 = 0.800, RSD = 89.6, n = 16).

The data corrected for the period effect are plotted in Figure 4.

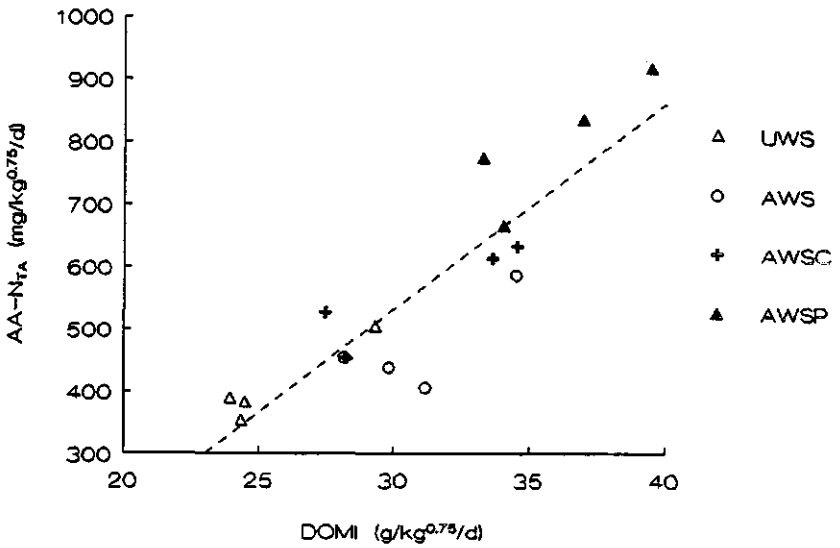

Figure 4. Relationship between truly small intestinally absorbed AA-N (AA-N_{TA}) and DOMI (data corrected for period effect).

The basis of the hypothesis that the amount of protein available for absorption from the small intestine determines intake of roughages could be that net energy and net protein availability to the tissues need to be balanced. DOMI could represent the net energy availability to the tissues. The conversion of DOMI to net energy occurs, for *ad libitum* fed rations, probably at a rather constant efficiency. The metabolizable energy content in digestible energy is approximately 0.80 without much variation between different feeds (Agricultural Research Council, 1980; Oosting et al., 1993c) and the efficiency of conversion of metabolizable energy to net energy for *ad libitum* rations fed to growing animals is also fairly constant (0.6) as postulated by Tolkamp and Ketelaars (1993). Also in the experiment reported by Oosting et al. (1993c) indications were found that metabolizable energy from *ad libitum* fed rations based on untreated and ammonia-treated wheat straw was utilized with an efficiency of 0.6 by sheep as well as by cattle. Regression of N-balance on DOMI gives therefore information about the balance between net protein and net energy availability to the tissues. In the present experiment, DOMI and N balance were linearly related. N balance was regressed on DOMI with inclusion of the period effect as a factor in the regression model. The following equation (intercept: mean over periods, between brackets s.e. of estimate) was found:

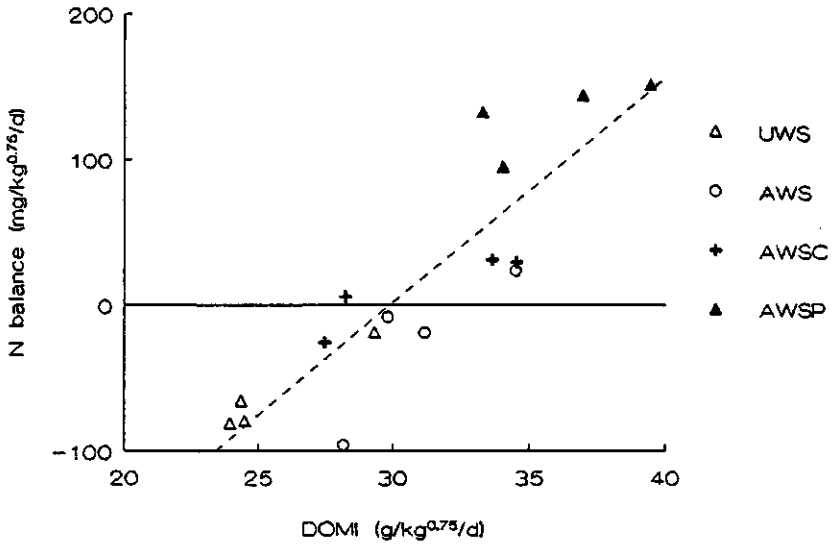

Figure 5. Relationship between N balance and DOMI (data corrected for period effect).

N balance (mg/kg$^{0.75}$/day) = -460 (81.0) + 15.4 (2.60) * DOMI (g/kg$^{0.75}$/day), R^2 = 0.773, RSD = 44.3, n = 16.

The data corrected for the period effect are plotted in Figure 5.

The equation predicts a zero N balance for a DOMI of 29.7 g/kg$^{0.75}$/day, higher than the maintenance requirements of 26 g DOMI/kg$^{0.75}$/day (ARC, 1980). Though the regression coefficient has a high s.e., it is remarkebly close to values observed by others for small ruminants fed roughage-based diets. Ketelaars and Tolkamp (1991) observed for West African Dwarf goats a value of 14.4 mg N balance/g DOMI. Recalculation of results of Elliott and Topps (1964), who measured TDN intake and N balance in Blackhead Persian sheep and Egan (1965) who measured digestible energy intake (DEI) in Merino sheep receiving dietary N supplements resulted in estimates of 14.6 and 15.3 mg N balance/g DOMI, respectively. TDN values given by Elliot and Topps (1964) were converted to DOM by accepting that 1 g TDN contains 0.95 g of DOM and the regression coefficient derived from regression of DEI on N balance as given by Egan (1965) was converted to the regression coefficient of N balance on DOMI by accepting 4.5 kcal DEI/g DOMI and that $b_{x,y} * b_{y,x} = R^2$. Larger regression coefficients (up to 20.9 mg N balance/g DOMI) were found

by Grenet and Demarquilly (1977) for Texel sheep. The linearity of these relationships between DOMI and N balance suggests that net protein and net energy availability to the tissues are balanced in *ad libitum* rations, hence that voluntary DOMI cannot increase without concomitant increase of net protein availability. However, Ørskov (1982) proposed that energy intake may increase without increased N balance, if N availability becomes limiting.

Rohr and Lebzien (1991) proposed as net efficiency of utilization of absorbed AA-N a value of 0.65. The maximum efficiency of utilization of absorbed protein for maintenance and production is determined by the supply of the most limiting essential amino acid. Requirements for individual amino acids depend on the relative contribution of processes as tissue maintenance, growth and endogenous losses to total amino acid utilization. As illustrated by the considerable differences in AAP between ileal endogenous protein losses and lean lamb meat (Table 7), different AAP's are required for different physiological processes. Hence, comparison of the AAP of truly absorbed protein with the AAP of lean lamb meat or milk as done by Van Bruchem et al. (1989) for identification of most limiting essential amino acids could at best be valid if requirements for tissue maintenance and endogenous losses are low proportional to total amino acid requirements.

The efficiency of utilization of AA-N_{TA} was estimated by regression of N balance on AA-N_{TA} with inclusion of the (significant) animal and period effect as factors in the regression model. The obtained regression equation was: (intercept averaged over animals and periods; between brackets s.e. of estimate)

N-balance (mg/$kg^{0.75}$/day) = -281 (15.9) + 0.54 (0.029) * AA-N_{TA} (mg/$kg^{0.75}$/day), R^2 = 0.989, RSD = 14.3, n = 16.

The data, corrected for the animal and period effect are plotted in Figure 6.
The intercept can be interpreted as the obligatory N losses as endogenous urinary N and metabolic faecal N. This value of 281 mg/$kg^{0.75}$/day is in the range of values varying from 201 to 427 mg/$kg^{0.75}$/day given by Ørskov (1982) for total urinary and faecal N excreted by fasting sheep or sheep maintained on N free diets. Estimates of the intercept for individual animals ranged from -242 to -323 mg/$kg^{0.75}$/day and for periods from -221 to -344 mg/$kg^{0.75}$/day. With increasing period number the value for the intercept increased, indicating that the maintenance requirements decreased with increasing duration of the experiment. The regression coefficient implies an utilization of truly absorbed AA-N of 0.54. This value is lower than 0.65, the value proposed by Rohr and Lebzien (1991). This could indicate that AA-N_{TA} was not utilized to the maximum extent, hence that AA-N_{TA} was not limiting N retention. As Figure 6 shows, there were, however, no indications of differences between rations with regard to the efficiency of AA-N_{TA} utilization, although it could be expected that AA-N_{TA} was more limiting for one ration than for the other. This suggests that 0.54 was the maximum efficiency of utilization of AA-N_{TA} for the sheep under the conditions in the present experiment.

Hence, if a balance is required between net protein and net energy availability in

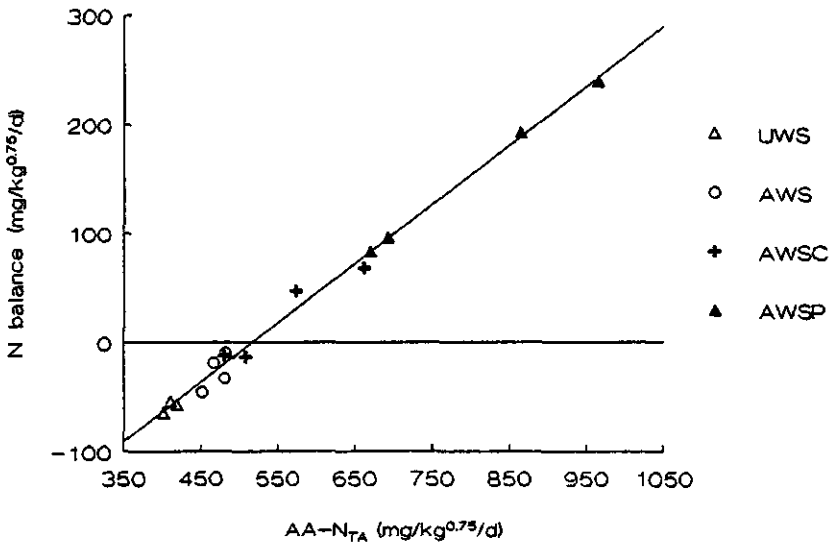

Figure 6. Relation between N balance and truly small intestinally absorbed AA-N (AA-N_{TA}) (data corrected for animal and period effect).

growing animals also a balance between DOMI and AA-N_{TA} seems required, because it is likely that conversion of DOMI to net energy and of AA-N_{TA} to net N retention occurs at constant efficiencies in *ad libitum* fed rations.

However, AA-N_{TA} and N balance are in part a function of DOMI. AA-N_{TA} from microbial origin and, to a lesser extent, originating from undegraded straw-protein increase with increasing straw DOMI. However, a calculation based on the results of ration AWSC shows that the increased AA-N_{TA} availability as a result of incremental straw DOMI is insufficient to achieve the ratio of 33.0 g AA-N_{TA}/kg DOMI (Figure 4) or 15.4 g N balance/kg DOMI (Figure 5). The efficiency of microbial protein synthesis for the diet with casein was 24 g microbial AA-N/kg ARDOMI. Of the total DOMI, 703 g/kg (Oosting et al., 1993b) was apparently degraded in the rumen. Hence, the microbial AA-N synthesis per kg DOMI was 16.2 g and the truly small intestinally absorbed quantity of microbial AA-N 14.0 g/kg DOMI. If animals fed AWSC would consume additional DOMI from straw, some additional AA-N_{TA} from straw origin would be available, but insufficient to achieve the quantity of 33.0 g/kg DOMI. Hence, it seems likely that the quantity of AA-N_{TA} produced as a function of DOMI was insufficient to balance nutrient availability at tissue level and supplementation with rumen undegradable protein was required to achieve increased DOMI.

Why limited protein availability to the tissues restricts voluntary intake is unknown. MacRae and Lobley (1982) and MacRae et al. (1985) reported that the efficiency of metabolizable energy utilization increased, and consequently the oxygen consumption per unit metabolizable or net energy ingested, with increasing protein availability. Tolkamp and Ketelaars (1992) postulated as a control mechanism for intake regulation that ruminants minimize oxygen consumption per unit net energy intake. These authors reported that the net energy intake level where the oxygen consumption per unit net energy ingested is minimal, increases with increasing efficiency of utilization of metabolizable energy. Hence, increased protein availability to the tissues could increase the efficiency of metabolizable energy utilization and consequently the voluntary intake.

Van Bruchem et al. (1989) related ileal endogenous amino acid losses to duodenal NPDM flow. For sheep fed roughages or roughage/concentrate mixtures they observed the following relation between ileal amino acid flow and duodenal amino acid and NPDM flow:

Ileal amino acid flow (mmol/day) = 59.9 + 0.152 * Duodenal amino acid flow (mmol/day) + 0.4134 * duodenal NPDM flow (g/day).

This relation implies a true small intestinal digestibility of amino acids of 0.848, which is approximately similar to the value of 0.86 observed in the present experiment and to the value of 0.85 observed by Storm et al. (1983) for amino acids of microbial origin. The major part of duodenal protein flow consists of microbial protein, which explains the similarity between results of various experiments, but it could be concluded from the present experiment that dietary amino acids added through potato protein supplementation were digested to the same extent as microbial protein. A high small intestinal digestion of N from dacron bags introduced into the duodenum and collected in faeces also indicated that N from the supplements was highly digestible in the lower gut. The small intestinal disappearances of N from dacron bags observed in the present experiment for untreated (652-774 g/kg) as well as for ammonia treated wheat straw (607-750 g/kg) were considerably higher than those for untreated (317 g/kg) and ammoniated barley straw (357 g/kg) as reported by Hvelplund (1989).

Sheep fed UWS had lower NPN losses per 100 g NPDM from the ileum than sheep fed rations based on ammoniated wheat straw. This could probably be attributed to the fact that part of the N added through ammonia treatment was not available for digestion. This is supported by the lower AA-N/N ratio in duodenal digesta for AWS and AWSC than for UWS. The results of Oosting et al. (1993a) also suggested that part of the N added through ammonia treatment could not be digested. Barnes (1988) observed by near infrared diffuse reflectance spectrometry that ammonia treatment leads to formation of amide groups with both cell wall and non cell wall components of wheat straw. It is likely that part of these amide groups are not digestible. However, as indicated by disappearance of N from dacron

bags over the whole digestive tract 800-900 g/kg of total N in AWS could potentially be digested, although it is possible that this could partly be attributed to solubilization.

Recalculation of the data of the experiment by Van Bruchem et al. (1989) by a model without intercept, yielded as estimate of ileal endogenous AA-N losses 7.9 mg/g NPDM for sheep fed roughage-based diets of a relatively high quality. Oosting et al. (1993a) estimated ileal endogenous AA-N losses as 5.0 mg/g NPDM for sheep fed UWS or AWS supplemented with sugar beet pulp, while in the present experiment ileal endogenous AA-N losses were estimated as 6.0 mg/g NPDM. This indicates that sheep fed straw based diets have lower ileal endogenous protein losses than sheep fed diets of higher quality with higher protein contents. Whether this is a result of a lower endogenous secretion in the distal part of the ileum or to higher re-absorption of secreted endogenous amino acids remains to be investigated.

Rumen microbes

No significant differences in composition of rumen microbes were found between rations. In an earlier experiment reported by Oosting et al. (1993a) differences in composition of rumen microbes were found between UWS and AWS. The efficiency of microbial protein synthesis (EMPS) in that experiment was (average of DAPA and AAP method) 24.6 and 19.0 g N/kg ARDOMI for UWS and AWS, respectively. In the present experiment EMPS (average of DAPA and AAP method) was 23.3 and 24.0 g N/kg ARDOMI for UWS and AWS, respectively. This indicates that in the earlier experiment nutrient supply may have been more limiting EMPS for AWS than in the present experiment. Compared with the earlier experiment, in the present experiment additional minerals including sulphur were supplied. Differences in nutrient availability for rumen microbes may affect microbial composition, either due to changes in contribution of various microbial strains to the microbial pool (Dufva et al, 1982) or to storage of polysaccharides in microbes (Czerkawski, 1986), which could explain the differences between the earlier and the present experiment.

The DAPA-N/N ratio's in the present experiment were in line with published data (Dufva et al., 1982; Siddons et al., 1982; Van Bruchem et al., 1985) and also the AAP's of microbial protein did not differ much from those reported elsewhere (Tas et al., 1981; Siddons et al., 1982; Van Bruchem et al., 1985 and 1988; Storm and Ørskov, 1983; Hvelplund and Hesselholt, 1987).

The concentration of rumen microbes in rumen fluid did not differ significantly between rations. Ration differences in fluid associated microbial pools could be attributed to differences in rumen fluid pools. The proportion of the total microbial pool associated with the fluid phase may vary from 20-47 % (Owens and Goetsch, 1986). Oosting et al. (1993a) observed for UWS as well as for AWS, that 36 % of the microbial pool was associated with the fluid phase.

The AAP method for estimation of proportions of dietary, microbial and endogenous AA-N in duodenal digesta is based on many assumptions which may not always be valid, as discussed by Siddons et al. (1982). However, estimates of rumen degradability of feed AA-N based on the AAP method were reasonably in line with data derived from the measurements of N disappearance from dacron bags incubated in the rumen. In addition, estimates of duodenal microbial AA-N flow based on AAP were comparable with those based on DAPA, though the AAP method gave a significantly higher estimate for EMPS for AWSC than did the DAPA method. Comparison of estimates of EMPS from the AAP and DAPA method reported in literature shows variable results. Both methods may gave similar results (Evans et al., 1975; Van Bruchem et al., 1985; Oosting et al., 1993a), AAP may give lower estimates than DAPA (Siddons et al., 1982; Voigt et al., 1991) or AAP may give higher estimates than DAPA (Van Bruchem et al., 1988). The method of estimation of duodenal microbial flow based on purine derivates gave results comparable with the AAP and DAPA method. The general trend with regard to the effect of rations on EMPS was that AWS had similar (DAPA and AAP) or higher (PD) EMPS than UWS and that AWSC and AWSP had higher EMPS than AWS. From this it may be concluded that the casein and potato protein supplements supplied nutrients, probably peptides and/or branched chain amino acids that were limiting microbial growth in AWS and UWS. Differences in EMPS between AWSC and AWSP were observed for the AAP method ($P < 0.05$) and the PD method ($P > 0.05$) suggesting that the quantity of rumen degradable true protein supplied through potato protein was insufficient to achieve maximum efficiency of microbial protein synthesis.

From the true rumen degradability of feed AA-N it was calculated that rumen ammonia-N contributed for 63, 58, 0 and 33 % to net microbial AA-N production for UWS, AWS, AWSC and AWSP, respectively. Oosting et al. (1993a) reported values of 59 and 67 % for UWS and AWS, respectively. These results indicate that in rations with a limited rumen true protein availability the maximum contribution of ammonia-N to microbial protein synthesis may approximate 60-70 %. The remaining fraction has to be provided by dietary true protein. The possible difference in EMPS of AWSC and AWSP even indicates that EMPS is higher when all microbial AA-N can be synthesized from dietary protein as with AWSC compared with AWSP, where 33 % of microbial AA-N originated from rumen ammonia.

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4.3 Effect of ammonia treatment of wheat straw with or without supplementation of potato protein on intake, digestion and kinetics of comminution, rumen degradation and passage in steers

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Abstract

Untreated (UWS) or ammoniated wheat straw, the latter without (AWS) or with a supplement of potato protein of a low rumen degradability (AWSP) was fed to three steers according to a 3 x 3 Latin square design. All rations were supplemented with sugar beet pulp and minerals. Voluntary organic matter intake (OMI, g/kg$^{0.75}$/day) was 67.8, 76.0 and 80.1 for whole rations (straw: 51.1, 59.7 and 59.2) for UWS, AWS and AWSP, respectively, significantly higher for AWS and AWSP than for UWS. Organic matter digestion (OMD, g/kg) was 561, 596 and 625 for the respective ration UWS, AWS and AWSP, also significantly higher for AWS and AWSP than for UWS. The increased voluntary intake and digestion for ammoniated wheat straw based rations were associated with a significantly higher potentially degradable fraction (D) of neutral detergent fibre (NDF) in offered straw (55.6 and 66.1 % for untreated and ammoniated wheat straw, respectively) and in the rumen pool (46.9, 55.5 and 55.4 % for UWS, AWS and AWSP, respectively). Isolated small rumen particles (retained on sieves with a pore size < 1.25 and > 0.041 mm) had a significantly lower D-fraction of NDF (average 58.8 %) than isolated large rumen particles (average 66.3 %). Fractional rates of degradation of NDF did not differ significantly either between untreated and ammonia treated wheat straw offered (2.9 and 2.6 %/h, respectively) or between rumen pools (1.8, 1.7 and 2.1 %/h for UWS, AWS and AWSP, respectively). Rations based on ammoniated wheat straw had a significantly higher rumen NH_3-N concentration than UWS. Though the rumen pool size of total contents differed significantly between treatments, those of dry and organic matter and of cell wall constituents were not significantly different. The proportion of rumen dry matter passing through a sieve with a pore size of 1.25 mm was averaged over rations 68.4 % (not significantly different between rations). Daily rumination (96 minutes) and eating (52 minutes) time per kg NDF ingested did not differ between rations. The rate of comminution of large particles, estimated from the disappearance of indigestible NDF in large particles from the rumen of animals without access to feed was 4.1, 6.3 and 7.1 %/h for UWS, AWS and AWSP, respectively. These values were not significantly different. The fractional rate of passage estimated from the faecal excretion of Cr-NDF was 5.4, 6.1 and 6.3 %/h for UWS, AWS and AWSP, respectively, (significantly higher for AWS and AWSP than for UWS) but the turnover rate of indigestible NDF did not differ between treatments.

Keywords: Ammonia treatment, wheat straw, rate of passage, rate of degradation, rate of comminution, rumen pool size.

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Introduction

Straws, which are important basal feeds for ruminants in densely populated areas in the tropics, are characterized by a low feed intake and digestibility. Ammonia treatment and/or supplementation with nutrients limiting to the animal or its rumen microbes are means to increase digestion and intake of the straw (Dias-da-Silva & Sundstøl, 1986; Cottyn & De Boever, 1988; Silva *et al.*, 1989; Mason *et al.*, 1990).

Intake of fibrous feeds and its regulation in ruminants is still far from sufficiently understood. Proposed regulation mechanisms vary from almost entirely metabolic to almost entirely physical. Recently, Ketelaars & Tolkamp (1992) and Tolkamp & Ketelaars (1992) proposed that feed intake is primarily governed by metabolic mechanisms optimizing the efficiency of energy utilization, thus keeping the noxious effects of oxygen radicals to a minimum and increasing longevity of the animal.

Alternatively, under temperate conditions Bosch *et al.* (1992) made an analysis of voluntary intake of roughage feeds in relation to the processing capacity of the reticulo-rumen as determined by its holding capacity or pool size and rate of feed turnover. The latter factor in turn comprises (1) the fractional rate of comminution of large particles into particles of a size which can potentially leave the reticulo-rumen, (2) the rate of fermentative degradation by the rumen microbes, and (3) the rate of passage of undegraded particles to the lower gut. For sheep and cattle, Kennedy & Poppi (1984) indicated a critical particle size (CPS) of 0.89 and 1.17 mm, respectively. In addition to that, the functional specific gravity of the feed particles is a parameter to be considered. Kaske *et al.* (1992) introduced particles of varying size and specific gravity in the rumen of sheep and concluded that in determining the probability of escape from the reticulo-rumen, the specific gravity of particles is superior to their size. Only small particles with a density equal to or higher than 1.22 showed a high probability of leaving the reticulo-rumen.

The present paper describes rumen pool sizes and kinetics of turnover in cattle fed low-quality fibrous feed as basal diet. The objective of this study was to assess the kinetics of rumen processing of the feed under conditions which usually prevail in the tropics. Knowledge of the magnitude of the parameters describing these various processes will allow the identification of those parameters which primarily constrain rumen processing capacity of low-quality fibrous feeds. Based on this information, a well documented approach could be designed aiming at improving ruminant production in the tropics on diets, largely constituted on highly fibrous feeds through the formulation of proper supplements in combination with chemical and/or physical treatment.

Material and methods.

Animals and diets

Three steers (breed Dutch Red and White) of about 500 kg live weight were fitted with a rumen cannula (Bar Diamond, 100 mm internal diameter) in the dorsal rumen sac. The steers were kept in tie stalls during the experiment and were fed twice daily at 07.30 and 19.30 h. Water was freely available.

The experiment was set up as a 3 x 3 Latin Square design with each experimental period lasting three weeks. Adaptation periods before each experimental period had a duration of three weeks. A time chart of measurements during each experimental period is given in Table 1.

Table 1. Time chart of activities during an experimental period

	Experimental week		
	1	2	3
Fasting from 9.00-18.00h		m t w	
Rumen evacuations	m t w	m t w	
Passage rates			
-k_1	m t	m t	
-$k_{p\text{-faeces}}$	t w t f	t w t f	
-$k_{p\text{-rumen}}$		m t w	
Particle size distribution			
- ingested straw		m t w	
- faeces	m		m
Chewing behaviour	m t w t f s		
pH, VFA and NH_3-N		f	
Intake and digestion		t f s	s m t w t f s
Dacron bag			t w t f s

s m t w t f s: Sunday up to Saturday

Treatments allocated to the animals were: 1) *ad libitum* wheat straw supplemented with sugar beet pulp (UWS), 2) *ad libitum* ammoniated wheat straw supplemented with sugar beet pulp (AWS) and 3) *ad libitum* ammoniated wheat straw supplemented with sugar beet pulp and potato protein (AWSP), a protein with a relatively low rumen degradability. *Ad libitum* intake was achieved by offering the animals at least 20 % surplus straw. The straw offered was chopped by a grass cutter to a length of approximately 5 cm. Ammonia treatment of the straw was done by anhydrous ammonia (40 kg per 900 kg straw dry matter). The ammonia was injected into a pile of baled straw covered with two sheets of polythene (0.15 mm thick). The duration of the treatment was seven weeks.

Sugar beet pulp, potato protein (Emsland-Stärke GmbH, Emlichheim, Germany) in case of AWSP, minerals and a commercially available mineral/vitamin mixture (Mervit 318; Premervo, Utrecht, The Netherlands) were offered as a pellet. The quantity of supplement dry matter (DM) offered for rations UWS and AWS was 18.2 g/kg$^{0.75}$/day and for ration AWSP 22.7 g/kg$^{0.75}$/day, thus achieving a DM intake (DMI) of sugar beet pulp and minerals/vitamins of 16.5 and 1.7 g/kg$^{0.75}$/day, respectively and an additional DMI of potato protein of 4.5 g/kg$^{0.75}$/day for AWSP. The proportions of the various constituents in the supplements are given in Table 2 and the chemical composition of the ration components is presented in Table 3.

Table 2. Composition of the supplements (g DM/kg DM).

	Sugar beet pulp	Sugar beet pulp + potato protein
Sugar beet pulp	904	713
Mervit 318 (Vit. A and D_3, trace elements)	45	36
$NaH_2PO_4.2H_2O$	32	25
$FeSO_4.7H_2O$	0.5	0.4
$MgSO_4.7H_2O$	19	15
Potato protein	nil	211

Table 3. Chemical composition of ration components.

	Untreated wheat straw	Ammoniated wheat straw	Sugar beet pulp	Sugar beet pulp + potato protein
DM (g/kg)	877	863	842	844
OM (g/kg DM)	920	920	907	920
NDF (g/kg DM)	797	786	301	250
ADF (g/kg DM)	505	509	158	130
ADL (g/kg DM)	65	60	13	12
N (g/kg DM)	6	11	21	46

Intake, digestion and dacron bag analysis

Intake and digestion were measured during a ten-day period in week two and three of each experimental period by total collection of feed residues and faeces. Urine was also collected and analysed for N. During week three, dacron bags (pore size 41 x 41 μm) with three to five gram (on dry matter basis) of straw, supplement, large rumen particles (size > 1.25 mm), small rumen particles (0.041 mm < size < 1.25 mm) and total rumen contents were incubated for 0.5, 6, 12, 24, 48, 72 and 96 h in the rumen of each steer in duplicate. Those ration components were incubated in each animal's rumen, that were included in its diet. In another rumen cannulated steer fed with wheat straw and sugar beet pulp the various samples were incubated for 336 h to determine the truly undegradable residue. Straw and supplements were ground through a 1 mm sieve, while the isolated large and small particles and total rumen contents were incubated as such. After removal of the bags from the rumen, they were washed in a washing machine for 30 minutes with cold water.

Rumen fermentation

Rumen fluid samples were taken during week two for analysis of diurnal fluctuations in pH, and NH_3-N and volatile fatty acid (VFA) concentrations. Rumen fluid samples were taken two hourly starting from 07.30 up to 19.30 h. The pH was measured immediately and samples for NH_3-N and VFA analysis were centrifuged and properly stored with TCA and 85 % phosphoric acid, respectively, pending further analysis.

Rumen evacuations

Rumen evacuations were done in the first and second week of each experimental period. Each steer's rumen was evacuated once daily over three days either at 09.30, 13.30 or 17.30 h. The rumen contents were weighed, sampled and the residue returned into the

rumen. This procedure took about 30 minutes per steer. During week one three percent of the rumen contents were taken: 1.5 % for separation into large (size > 1.25 mm) and small (0.041 mm < size < 1.25 mm) particles and subsequent incubation in dacron bags, 0.5 % for incubation in dacron bags, 0.5 % for wet sieve analysis and 0.5 % for analysis of composition.

During week two feed was withdrawn from the animals from 9.00 till 18.00 h during the three days when evacuations took place. Two percent of the rumen contents were sampled: One percent for wet sieve analysis and one percent for analysis of composition including Cr. The rate of turnover of large and small particles was measured from the logarithmic decline in rumen large and small particle pool.

Particle size distribution

Particle size distribution of straw immediately upon ingestion and chewing was measured in week two by allowing the animals to consume straw during 15 minutes, while having an empty rumen. The ingested material was removed from the rumen and as such stored in a freezer pending sieve analysis.
Faecal samples for analysis of particle size distribution were taken once in week one and once in week three.

Wet sieve analysis of rumen and faecal samples was done on a Fritsch Analysette 3. The procedure was as described in detail by Bosch *et al.* (1993b). For faecal samples the mesh apertures of the sieves used were 1.25, 0.63, 0.315, 0.16 and 0.071 mm. For rumen samples the mesh apertures were 5, 2.5, 1.25, 0.63 and 0.071 mm. Rumen samples obtained immediately after ingestion were sieved through a sieve of 1.25 mm. Separation of rumen samples into large and small particles for incubation in dacron bags was done through sieves of 1.25 mm and dacron cloth with a pore size of 0.041 mm. Hence, small particles passing this dacron cloth were not included in the isolated small particles. Particles retained on sieves with a diameter larger than 1.25 are referred to as large particles (LP), while particles passing that sieve are considered small particles (SP).

The mean particle size was calculated using a logarithmic transformation of the sieve diameter as described by Waldo *et al.* (1971). It was assumed that material passing the 0.071 mm sieve should be retained on a 0.001 mm sieve.

Chewing behaviour

During week one the eating and rumination behaviour of the animals was recorded on video tape for each animal during two days. Time was also recorded on the video tapes. From these recordings the daily time spent eating and ruminating and the number of chews per minute rumination were obtained. The latter was done by counting the number of chews during one minute rumination for five rumination periods daily.

Passage rates

During the first and second week of each experimental period the fractional passage rates of the liquid and particulate phases in the reticulo-rumen were estimated with Co-EDTA and Cr-NDF, respectively (prepared according to Udén *et al.*, 1980). At day one at 06.30 h, 100 g of Cr-NDF (4.9 % Cr, particle size 0.2-1 mm) and 30 g dry Co-EDTA (15.0 % Co) were administered into the ventral rumen sac. Rumen fluid samples were collected every two h from 07.30 up to 19.30 h at day one and from 07.30 up to 11.30 h at day two. Faecal samples for Cr analysis were collected over the following time intervals: at day two: 07.30-11.30, 11.30-15.30 and 15.30-19.30 h; at day three and four: 19.30-07.30, 07.30-11.30, 11.30-15.30 and 15.30-19.30 h and at day five : 19.30-07.30, 07.30-13.30 and 13.30-19.30 h. Fractional rate of passage of the liquid phase (k_l) was derived from the logarithmic decline of the rumen Co concentration and the fractional rate of passage of the particulate phase was estimated from the declining phase of the logarithmically transferred faecal excretion curve of Cr ($k_{p\text{-faeces}}$, peak concentration defined after plotting). No correction was made for Co and Cr withdrawal from the rumen by sampling during rumen evacuations.

The fractional rate of passage of the particulate phase was also estimated from the logarithmic decline in rumen Cr pool ($k_{p\text{-rumen}}$) as estimated in animals which had no access to feed from 09.00-18.00 h during week 2.

Chemical analysis

Offered and residual feed, faeces and rumen samples were analysed for dry matter (DM), ash, nitrogen (N), neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL). Large and small rumen particles and dacron bag residues were analysed for DM, ash and NDF. Analysis of rumen samples was done after freeze drying, while analysis of all other samples was done after drying at 70°C. Dry matter was determined by drying at 103 °C, ash by ashing at 550 °C and N by the Kjeldahl method. Cell wall analysis was done according to Goering & Van Soest (1970). Hemicellulose was calculated as NDF minus ADF and cellulose as ADF minus ADL. Co in rumen fluid samples and Cr in faeces were determined by atomic absorption spectrophotometry (Varian SpectraA 300, wave length 240.7 nm for Co and 357.9 nm for Cr) after wet destruction. NH_3-N in rumen fluid was measured by the indophenol method (Scheiner, 1976) with a Hitachi u2000 spectrophotometer at a wavelength of 634.8 nm. VFA's were measured by gas liquid chromatography (Packard 419, glass column (6 feet, internal diameter 2 mm) filled with Chromosorb 101, 80-100 mesh, carrier gas (N_2) saturated with formic acid, 190 °C).

Calculations and statistics

Rates of degradation were calculated according to the model of Robinson et al. (1986):

(1) $R_t = U + D*e^{-kd*(t-t')}$ for $t \geq t'$

in which:

R_t = residue (%) at time t
U = truly undegradable fraction (%)
D = potentially degradable, but water insoluble fraction (%)
k_d = fractional rate of degradation (%/h) of fraction D.
t' = lag time (h)

The model was fitted by the non-linear regression procedure of the statistical program DBSTAT (Brouwer, 1989). The lag time was estimated as part of the model. For rumen samples no lag time was included in the model.

The rate of comminution (k_c) of large particles was calculated from the logarithmic decrease of the rumen large particle NDF pool in fasting animals and the rumen degradation characteristics of NDF in large rumen particles by the formulas:

(2) $\ln(LP_t) = \ln(LP_0) - k_{cl} * t$, where LP_t is the size of the rumen large particle NDF pool at time = t and k_{cl} is the rate of clearance of the rumen large particle NDF pool.

(3) $k_c = k_{cl} - k_{d(LP)} * D/(D + U)$ where $k_{d(LP)}$, D and U are rate of degradation, the potentially degradable and the truly undegradable fractions of the rumen large particle NDF pool, respectively.

The data were analysed statistically using the anova procedure of the DBSTAT program (Brouwer, 1989). The data were analyzed according to the model

(4) $Y_{ijkl} = \mu + Period_i + Animal_j + Treatment_k + error_{ijkl}$.

Total degrees of freedom (d.f.) were 9 and each of the factors and the error had d.f. = 2. Significance of differences between ration means were tested by Student's t-test.

Results

Intake and digestion

Intake and digestion of whole rations are presented in Table 4. Ammonia treatment increased intake of organic matter (OM), NDF and cellulose and N significantly. The organic matter intake (OMI) of straw was 51.1, 59.7 and 59.2 g/kg$^{0.75}$/day for UWS, AWS and AWSP respectively (SEM 1.18), significantly higher for AWS and AWSP than for UWS.

Straw OMI increased by 16 % due to ammonia treatment. No significant effect of supplementation of potato protein to ammoniated wheat straw on intake of any nutrient, except N was observed.

Digestion of OM, NDF, hemicellulose, cellulose and N was significantly higher for AWS than for UWS. Ammonia treatment of wheat straw increased the digestion of cellulose and hemicellulose to about the same order, by 13 % for hemicellulose and by 12 % for cellulose. AWSP had a significantly higher digestion of hemicellulose and N than AWS. Analysis of N digestibility by the following Lucas equation (van Soest, 1982):

(5) $DNI = - a + b * NI$,

in which DNI is the digestible N intake as percentage of OMI, a is the intercept interpretable as the metabolic faecal N excretion (g) per 100 g OMI, b is the true digestibility of N and NI is the N intake (% of OMI), yielded as estimates for metabolic faecal N excretion 0.61 (SE 0.079) g/100 g OMI and for true N digestibility 0.88 (SE 0.051) ($R^2 = 0.973$, RSD = 0.067, n = 9).

Assuming an organic matter digestion (OMD) for the sugar beet pulp supplement of 850 g/kg the estimated straw OMD was 467, 525 and 545 g/kg for UWS, AWS and AWSP, respectively (SEM 7.2), significantly higher for AWS and AWSP than for UWS. Ammonia treatment of wheat straw increased straw OMD by 12 %.

DOMI (digestible organic matter intake) was significantly higher for AWS than for UWS, while DOMI for AWSP was significantly higher than for AWS.

N retention was significantly higher for AWSP than for AWS and UWS. The apparent utilization of digested N, calculated as N retained/N digested did not differ significantly between rations and was 226, 151 and 273 g/kg for UWS, AWS and AWSP, respectively (SEM 57.7).

Table 4: Intake, digestion, N retention and average weight of animals.

	UWS	AWS	AWSP	SEM
Intake ($g/kg^{0.75}$/day)				
OM	67.8^a	76.0^b	80.1^b	1.11
NDF	49.8^a	56.4^b	56.4^b	1.12
Hemicellulose	18.8	20.6	20.5	0.51
Cellulose	27.1^a	31.8^b	31.8_b	0.75
ADL	3.9	4.1	4.1	0.06
N	0.72^a	1.14^b	1.77^c	0.001
Digestion (g/kg)				
OM	561^a	596^b	625^b	4.9
NDF	525^a	595_b	617^b	4.8
Hemicellulose	587^a	664^b	696^c	2.8
Cellulose	605^a	676^b	691^b	8.9
N	378^a	458^a	613^c	16.2
DOMI ($g/kg^{0.75}$/day)	38.1^a	45.3^b	50.1^c	0.40
N retained ($mg/kg^{0.75}$/day)	68^a	81^a	299^b	27.2
Average weight (kg)	499	493	507	2.80

Different superscripts in a row indicate significant differences ($P < 0.05$).

In Table 5 the degradation characteristics of the ration components are given. Only those ration components were incubated in the rumen of each animal that were included in its diet. Because no differences were found in degradation parameters of ammoniated wheat straw incubated in the rumen of steers fed AWS or AWSP the data for ammoniated wheat straw were pooled. Also the data for sugar beet pulp were pooled, since no significant difference was found between sugar beet pulp incubated in the rumen of steers fed UWS or AWS.

The potential degradable fraction (D) of DM, OM and NDF was significantly higher and the truly undegradable fraction (U) and the lag time (t') significantly lower for AWS than for UWS. The apparent solubility of DM, OM and NDF, which is equal to 100 - D - U did not differ between UWS and AWS. This apparent solubility of NDF was approximately similar to that of OM. Since NDF is by definition insoluble, the apparent solubility should be attributed to escape of small particles from the bags. Inclusion of potato protein in the sugar beet pulp pellet had no significant effect on DM and OM degradation parameters.

Table 5. *In sacco* degradation parameters of ration components. Between brackets SEM. (D: potentially degradable fraction, %; U: truly undegradable fraction, %; k_d: rate of degradation, %/h; t': lag-time, h).

		Untreated wheat straw (n = 3)	Ammoniated wheat straw (n = 6)	Sugar beet pulp (n = 6)	Sugar beet pulp + potato protein (n = 2)
DM	D	49.9^a (2.75)	61.2^b (1.94)	52.4 (1.00)	56.7 (1.73)
	U	33.9^a (1.68)	23.5^b (1.19)	4.0 (1.06)	5.5 (1.83)
	k_d	2.7 (0.23)	2.3 (0.16)	5.7 (0.18)	6.2 (0.32)
	t'	12.3^a (1.43)	3.0^b (1.01)	nd	nd
OM	D	51.4^a (2.96)	63.1^b (2.09)	55.5 (1.01)	59.8 (1.75)
	U	34.5^a (1.86)	24.1^b (1.32)	3.6 (0.96)	4.9 (1.66)
	k_d	2.9 (0.15)	2.5 (0.11)	5.7 (0.17)	6.1 (0.30)
	t'	13.1^a (1.11)	3.3^b (0.79)	nd	nd
NDF	D	55.6^a (2.20)	66.1^b (1.56)		
	U	27.0^a (1.40)	18.5^b (0.99)		
	k_d	2.9 (0.18)	2.6 (0.13)		
	t'	13.3^a (0.76)	4.0^b (0.54)		

Different superscripts in a row indicate significant differences between untreated and ammoniated wheat straw ($P < 0.05$).
nd: not determined.

Degradation characteristics of rumen contents

D- and k_d-values of NDF in isolated large (LP) and small (SP) rumen particles are given in Table 6. Since the particles were isolated by wet sieving through sieves of 1.25 mm and 0.041 mm for LP and SP, respectively, in this case no significant escape from dacron bags of particles at t = 0 was observed. The average NDF disappearance for LP and SP at t = 0 was only 0.92 % (SEM 0.436), without any significant differences between rations and between LP and SP. D- and k_d-values of SP and LP did not differ significantly between UWS and AWS. Supplementation of AWS with potato protein did not affect D in SP or LP, but k_d of SP increased significantly, though slightly.

The D-fraction of the whole rumen NDF pool was significantly higher and the U-fraction significantly lower for AWSP and AWS than for UWS. The apparent solubility of the whole NDF pool, which is assumed to be escape of small particles from the dacron bags, was 16.8, 16.0 and 16.2 % for UWS, AWS and AWSP, respectively. The k_d-value of the whole rumen NDF pool was not significantly different between rations.

The difference in D-fractions between SP and LP was over rations significantly lower than zero, indicating, that D in SP was significantly lower than in LP. No significant differences between k_d-values of SP, LP and the whole rumen NDF pool were found.

Table 6. Potentially degradable fraction (D) and rate of degradation (k_d) of small (SP) and large (LP) NDF particles and D, k_d and truly undegradable fraction (U) of the whole rumen NDF pool.

		UWS	AWS	AWSP	SEM
SP[1]	D (%)	55.4	61.9	59.2	1.93
	k_d (%/h)	1.8[a]	1.8[a]	2.0[b]	0.02
LP[1]	D (%)	58.7	69.1	71.2	3.20
	k_d (%/h)	1.5	2.2	2.0	0.19
NDF-pool	D (%)	46.9[a]	55.5[b]	55.4[b]	1.08
	U (%)	36.3[a]	28.5[b]	28.4[b]	1.25
	k_d (%/h)	1.8	1.7	2.1	0.17

Different superscripts in a row indicate significant differences ($P < 0.05$).
[1] U = 100 - D.

Rumen fermentation

The diurnal variation in NH_3-N concentration in the rumen fluid is illustrated in Figure 1. The ammonia-N concentration in rumen fluid of steers fed AWS or AWSP increased during the first hours after feeding and decreased gradually thereafter. The diurnal variations in pH and rumen VFA concentration were low.

Average daily rumen pH, NH_3-N and VFA concentrations are given in Table 7. UWS had a significantly lower NH_3-N concentration than AWS and AWSP. No significant differences either in VFA concentration in the rumen fluid or in molar proportions of individual VFA's were found between rations.

Rumen pool size

Table 8 shows the rumen pool sizes in steers that had access to the feed during the whole day. The total rumen pool size was gradually declining between the morning and evening feeding and was 82.6, 78.2 and 74.2 kg at 9.30, 13.30 and 17.30 h, respectively.

Ammoniation of wheat straw reduced total rumen pool size significantly and supplementation of potato protein to AWS significantly increased the weight of rumen contents compared to AWS. Scaled to liveweight UWS had a significantly higher rumen fill than AWS, while no significant difference between AWS and AWSP was found. However, no differences in pool sizes of DM, OM and cell wall constituents were observed between rations. The mean DM concentration in the rumen pool was 103, 118 and 109 g/kg (SEM 5.2) for UWS, AWS and AWSP, respectively, not significantly different between rations.

Also DM pools of large and small particles did not differ between rations. Small

particles contributed for 69.1, 67.2 and 68.8 % (SEM 1.42) to the total rumen DM pool for UWS, AWS and AWSP, respectively. Since the NDF content of LP and SP did not differ (respectively 681 and 688 g/kg DM) the contribution of NDF in SP to the rumen NDF pool was equal to the contribution of DM in SP to the rumen DM pool. Mean rumen particle sizes calculated according to Waldo et al. (1971) were 175, 176 and 176 μm for UWS, AWS and AWSP, respectively (SEM 8.3, ns). Relative contributions of particles retained on sieves with mesh apertures of 1) in between 1.25 and 0.63 mm, 2) in between 0.63 and 0.071 mm and 3) smaller than 0.071 mm to the small particle DM pool were averaged over rations (between brackets SEM) 216 (38.1), 413 (68.7) and 363 (66.7) g/kg, respectively. This particle size distribution within the rumen SP pool did not differ significantly between rations.

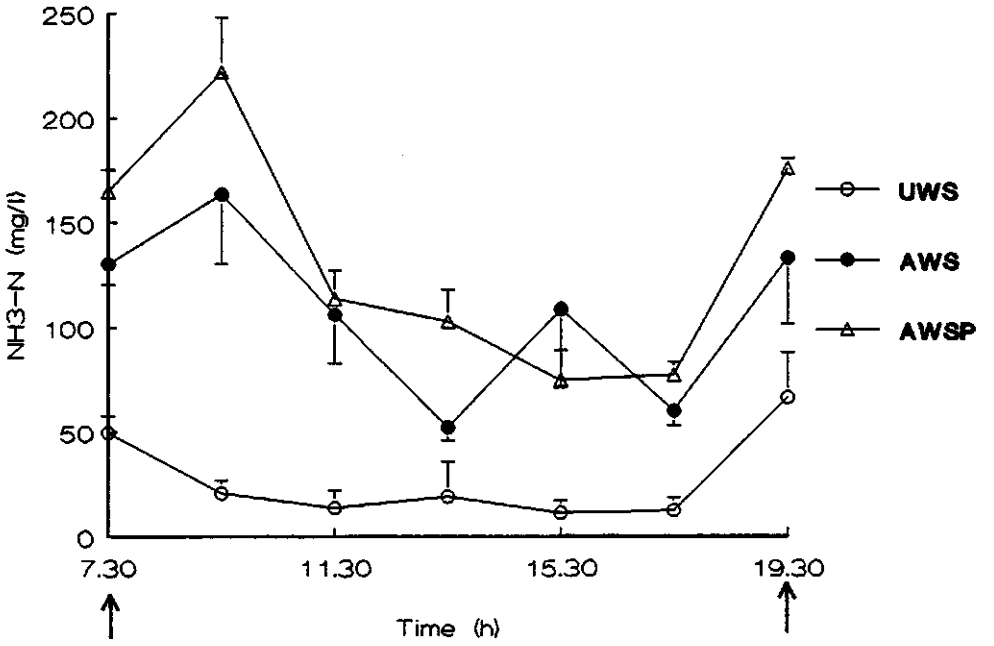

Figure 1. Diurnal variation in NH_3-N concentration in rumen fluid. Arrows indicate feeding time.

Table 7. Average pH, NH_3-N and VFA concentration in rumen fluid and molar composition of VFA's (averages from 09.30 up to 19.30 h).

	UWS	AWS	AWSP	SEM
pH	6.61	6.59	6.52	0.026
NH_3-N (mg/l)	21.1[a]	103.4[b]	125.9[b]	4.83
VFA (mmol/l)	81.5	85.8	87.7	4.18
Molar proportions (mol/100 mol):				
HAc	73.9	74.7	74.7	0.75
HPr	16.8	16.3	15.7	0.57
HBu	8.9	8.7	9.1	0.25
HVa	0.4	0.3	0.4	0.02

Different superscripts in a row indicate significant differences ($P < 0.05$).

Table 8. Rumen pool sizes.

	UWS	AWS	AWSP	SEM
Total contents				
- kg	80.5[a]	76.3[b]	78.1[c]	0.22
- % of live weight	16.1[a]	15.5[b]	15.4[b]	0.07
DM				
- pool > 1.25 mm (kg)	2.55	2.94	2.65	0.184
- pool < 1.25 mm (kg)	5.70	6.03	5.81	1.040
- total (kg)	8.24	8.97	8.45	0.970
OM (kg)	7.31	8.09	7.54	0.794
NDF (kg)	5.79	6.10	5.72	0.660
Hemicellulose (kg)	2.18	2.19	2.07	0.264
Cellulose (kg)	3.00	3.24	3.05	0.324
Lignin (kg)	0.62	0.67	0.59	0.073

Different superscripts in a row indicate significant differences ($P < 0.05$).

Chewing behaviour

Daily eating and rumination time is given in Table 9. No significant differences emerged between rations with regard to total chewing and eating time, either in absolute terms or when scaled to NDFI or DOMI. Rumination time was significantly higher for AWSP than for UWS, but the difference between rations disappeared when rumination time was scaled to NDFI. The number of chews per minute rumination did not differ between rations.

Table 9: Daily total chewing, eating and rumination time.

	UWS	AWS	AWSP	SEM
Total chewing time				
- min./day	833	830	849	23.3
- min./kg NDFI	161	141	141	9.7
- min./kg DOMI	210	176	159	11.1
Eating				
- min./day	319	288	275	24.0
- min./kg NDFI	62	49	46	6.5
- min./kg DOMI	81	61	51	7.4
Rumination				
- min./day	514^{a}	541^{ab}	574^{b}	6.5
- min./kg NDFI	99	92	96	3.4
- min./kg DOMI	130	115	108	4.2
Chews/min. rumination	60.7	60.4	59.6	0.57

Different superscripts in a row indicate significant differences ($P < 0.05$).

Comminution of large particles

Kennedy & Poppi (1984) defined the critical particle size (CPS) as the sieve aperture retaining the top 5 % of faecal particulate DM. For cattle they proposed 1.17 mm as CPS. In the present experiment, the 1.25 mm sieve retained on average 4.3, 4.9 and 4.8 % of the faecal particulate DM for the rations UWS, AWS and AWSP, respectively.

The rate of clearance of LP, SP, and the rate of comminution (k_c) in animals without access to feed and the mean faecal particle size are given in Table 10. The rate of clearance of the LP pool was significantly higher for AWSP than for UWS. Supplementation of potato protein to AWS did affect neither the turnover nor the comminution rates significantly. The turnover rate of SP was significantly lower than that of LP.

Mean faecal particle size was calculated according to Waldo et al. (1971) and was significantly reduced by ammonia treatment. Potato protein supplementation did not affect the mean faecal particle size compared to AWS.

Table 10. Rates of clearance of rumen pools of small and large NDF particles, rate of comminution of large NDF particles and mean faecal particle size.

	UWS	AWS	AWSP	SEM
Clearance rate of SP - pool (%/h)	1.1	0.5	0.8	0.17
Clearance rate of LP - pool (%/h)	5.0[a]	7.8[ab]	8.7[b]	0.59
k_c (%/h)	4.1	6.3	7.3	0.59
Mean faecal particle size (μm)	75.1[a]	61.9[b]	58.0[b]	1.57

Different superscripts in a row indicate significant differences ($P < 0.05$).

The distribution of particles size immediately upon ingestion was measured in cattle with an emptied rumen. The animals were allowed to eat straw for 15 minutes and the ingesta were removed immediately after swallowing. Only four samples could be obtained, 2 of UWS and 2 of AWS. On the other occasions the animals refrained from eating. The average LP fraction for ingested feed was 56.2 % for UWS and 45.6 % for AWS (values not significantly different; SEM = 4.71).

Rates of passage of fluid and particulate phases.

The fractional flow rate of rumen liquid (k_l) and particulate phases measured either from the decline in marker concentration in faeces ($k_{p\text{-faeces}}$) or from the decline in rumen pool of Cr ($k_{p\text{-rumen}}$) are given in Table 11. In this table is indicated whether the estimates were derived from fasting animals (feed withdrawn from 09.00-18.00 h during the first three days after introduction of the markers into the rumen) or non fasting animals. The k_l, $k_{p\text{-faeces}}$ and $k_{p\text{-rumen}}$ were lower for UWS than for the other rations. The difference between fasting and non fasting was over rations significantly different from zero for k_l and $k_{p\text{-faeces}}$, suggesting, that k_l was significantly higher and $k_{p\text{-faeces}}$ significantly lower in fasting than in non fasting animals. The $k_{p\text{-rumen}}$ measured during fasting was over and within rations significantly lower than the $k_{p\text{-faeces}}$ measured during fasting.

The rates of turnover of the rumen indigestible NDF ($k_{p\text{-iNDF}}$) and lignin ($k_{p\text{-lignin}}$) pools were calculated as intake/rumen pool size. For estimation of the rate of turnover of the rumen iNDF pool ($k_{p\text{-iNDF}}$) the following assumptions were made:

(1) the U-fractions of straw NDF intake were estimated as U/(D+U) (see Table 5), which means, that the ratio U/D of the apparently soluble part was assumed equal to

Table 11. Fractional rate of passage of liquid phase (k_l), particulate phase derived from faecal Cr excretion ($k_{p\text{-faeces}}$) or decreasing rumen Cr pool during fasting ($k_{p\text{-rumen}}$), rumen turnover (intake/rumen pool size) of iNDF ($k_{p\text{-iNDF}}$) and of lignin ($k_{p\text{-lignin}}$).

	UWS	AWS	AWSP	SEM
k_l (%/h)				
- non fasting	8.8^a	10.4^b	9.6^{ab}	0.18
- fasting[1]	9.2^a	10.4^b	10.3^b	0.13
$k_{p\text{-faeces}}$ (%/h)				
- non fasting	5.4^a	6.1^b	6.3^b	0.12
- fasting[1]	4.9	5.7	6.0	0.19
$k_{p\text{-rumen}}$ (%/h)				
- fasting[1]	4.5^a	5.4^b	5.2^{ab}	0.12
$k_{p\text{-iNDF}}$ (%/h)				
- non fasting	2.6	2.4	2.5	0.48
$k_{p\text{-lignin}}$ (%/h)				
- non fasting	2.8	2.7	3.1	0.45

Different superscripts in a row indicate significant differences ($P < 0.05$).
[1] animals fasting from 09.00-18.00 h during the first three days after introduction of the marker.

that of the part that remained in the bags,

(2) the U-fractions of NDF of the supplements were equal to the U fraction of OM (see Table 5),

(3) the U-fractions of the rumen NDF pool were the weighted means (based on the proportion of particles in the rumen) of the U-fractions of isolated SP and LP (see Table 6). Since particles, that were not retained on dacron cloth with a pore size of 0.041 mm were not included in the fraction of isolated SP, the assumption was, that these very small particles had the same U-fraction as the isolated SP.

The $k_{p\text{-iNDF}}$ and $k_{p\text{-lignin}}$ did not differ significantly between rations. Over rations, $k_{p\text{-lignin}}$ minus $k_{p\text{-iNDF}}$ was significantly higher than zero, indicating that the rate of turnover of iNDF was significantly lower than that of lignin. If the rate of turnover of the small particle iNDF pool was calculated as iNDFI/rumen small particle iNDF pool, the derived values were 3.7, 3.6 and 3.3 %/h for UWS, AWS and AWSP, respectively (SEM 0.844, ns). The $k_{p\text{-rumen}}$ and $k_{p\text{-faeces}}$ refer to the rumen small particle pool, since they were determined with Cr-NDF particles of a size smaller than 1.25 mm. Both $k_{p\text{-rumen}}$ and $k_{p\text{-faeces}}$ were significantly higher than the rate of turnover of the small particle iNDF pool.

Discussion

Intake, digestion and composition

Intake and digestion of wheat straw increased due to treatment with anhydrous ammonia. Intake of straw OM increased by 16 % and digestion of straw OM increased by 12 %. These increases are comparable to results of other experiments in which untreated and ammoniated wheat or barley straws were compared (Dias-da-Silva & Sundstøl, 1986; Cottyn & De Boever, 1988; Silva *et al.*, 1989; Mason *et al.*, 1990). However, Zorrilla-Rios *et al.* (1991) observed only an effect of ammonia treatment of wheat straw on in vitro DMD, but not on in vivo OMD.

The effect of alkaline treatment of straws is attributed to the disruption of ester bonds between hemicellulose and aromates, which can be found in the lignin portion (Morrison, 1983; Chesson *et al.*, 1983; Mason *et al.*, 1990). Morrisson (1983) found after delignification a higher increase in digestion of hemicellulose than of cellulose. This was also found by Dias-da-Silva & Sundstøl (1986) for ammonia treated wheat straw fed to sheep. In the present experiment approximately a similar increase in digestion of hemicellulose and cellulose after ammonia treatment was found as also observed by Ternrud *et al.* (1987).

Ammonia treatment often results in a reduction of the cell wall fraction in straw DM (Ørskov *et al.*, 1989; Dias-da-Silva & Sundstøl, 1986; Zorrilla-Rios *et al.*,1991), as a result of a reduction in hemicellulose concentration (Zorrilla-Rios *et al.*, 1991; Dias-da-Silva & Sundstøl, 1986). Others, however, only found a marginal reduction in cell wall concentration (Mason *et al.*, 1990) or hemicellulose concentration (Ternrud *et al.*, 1987). In the present experiment, the hemicellulose concentration decreased only marginally from 292 g/kg to 277 g/kg.

Energy intake from low quality feeds may be limited by small intestinal availability of protein (Egan, 1977; Doyle & Panday, 1990). Increased small intestinal protein supply through supplementation with potato protein of a low rumen degradability had no significant effect on OMI and OMD of ammoniated wheat straw in the present experiment. Total DOMI increased, however, and no substitution of straw intake by protein intake occurred.

The incremental intake of wheat straw as an effect of ammonia treatment has to be processed in the rumen. Ingested feed is either degraded in the rumen or passes undegraded from the rumen. Degradation in the rumen is a function of the fractional rate of fermentative degradation and the pool size of potentially degradable material. Microbial degradation in the rumen can be limited by N or true protein availability (Hespell & Bryant, 1979). Passage from the rumen is related to the rate at which large particles (unable to leave the rumen) are comminuted to small particles and to the rate of passage of small particles. In this respect the functional specific gravity of particles plays a predominant role (Kaske *et al.*, 1992).

Whether parameters related to rumen processing could explain the effects of ammonia treatment on intake and digestion of wheat straw will be discussed in the following sections. In addition, results from the present experiment were compared with those from experiments

with dairy cattle of 500-550 kg during late lactation reported by Bosch *et al.* (1993a, 1993b). These cattle consumed wilted grass silages *ad libitum* and received 1 kg concentrate. NDF digestion of the four silages in these experiments was on average 714 g/kg (range 629-780 g/kg) and NDFI 60.3 g/kg$^{0.75}$/day (range 49.6-69.5 g/kg$^{0.75}$/day), both higher than for the straws in the present experiment.

Rumen degradation

Ammonia treatment increased the D-fraction and decreased the U-fraction of wheat straw. Untreated wheat straw in the present experiment had a lower U-fraction than reported by Von Keyserlingk & Mathison (1989), who incubated up to 120 hours, whereas ammonia treated and untreated wheat straw had higher U-fractions than reported by Ørskov *et al.* (1989).

The D-fractions of ingested straw's NDF were approximately similar to that of rumen large NDF particles, while these large NDF particles had a significantly higher D-fraction than small NDF particles. This could be explained by the fact that the average retention time in the rumen was shorter for large particles than for small particles. The D-fractions of the whole rumen NDF pool was significantly higher for ammonia treated straw based rations than for UWS.

The lag time was reduced due to ammonia treatment, which could be related to the better rumen environment (N availability) in which the bags were incubated and a faster hydration and microbial attachment and subsequent colonization due to removal of the waxy surface layer by ammonia treatment.

The fractional rate of degradation was not significantly affected by ammonia treatment as is also apparent from the data of Ørskov *et al.* (1989). The rate constants of degradation of the rumen NDF pool were lower than those of ingested straw. This indicates that the absolute value of k_d measured in the feed cannot be applied as such to the rumen contents. Aitchisson *et al.* (1986) concluded that estimates of k_d derived from dacron bag incubations underestimate actual k_d values and are at best relative indicators of the rate at which degradation occurs.

D and k_d of NDF in wilted grass silages incubated in cattle during late lactation (Bosch *et al*, 1993a) were on average 82 % (range 70-89 %) and 4.5 %/h (range 2.7-6.4), respectively. Comparison with values of 78 % for D/(D+U) and 2.6 %/h for k_d of ammoniated wheat straw, indicates that ammoniated wheat straw is inferior to wilted grass silage, mainly in respect of k_d of NDF .

The rate of microbial degradation in the rumen could be affected by rumen environment. Ammoniation of wheat straw had no effect on rumen pH, rumen VFA concentration and molar proportions of individual VFA's. A rumen NH_3-N concentration for maximum *in vitro* degradation of low quality roughages in the range of 60-100 mg/l was reported by Oosting *et al.* (1989). Hence, the rumen NH_3-N concentration was lower than required for UWS, while for AWS and AWSP the rumen NH_3-N concentrations were above

or in the required range between the morning and evening feeding. However, the similarity of k_d-values between straws incubated in different rumen environments suggests, that rumen NH_3-N availability might not have limited rumen degradation for UWS. Despite the small, but significant difference in k_d of SP between AWS and AWSP, the conclusion seems also justified that rumen true protein availability was not limiting k_d of ammoniated wheat straw either.

Rumen degradation of NDF in a steady state situation can be described as $k_d/(k_p+k_d)*(D/(D+U))$ (Aitchisson *et al.*, 1986). The k_d-values were not different between treatments and accepting similar k_p-values for the rations, the foregoing formula predicts a higher rumen degradation (in line with the whole-tract observations) for ammonia-treated wheat straw-based diets than for UWS as an effect of the higher D content of the rumen NDF pool.

Passage from the rumen

Passage of particles from the rumen is restricted to small particles only and the rumen small particle pool size could limit passage. The relatively high proportion of small particles in the rumen observed for all rations indicates, however, that passage of particles from the rumen was not limited by comminution rate. Bosch *et al.* (1993b), who observed that the proportion of small particles in the rumen DM of dairy cattle fed wilted grass silages was higher than 70 %, arrived at a similar conclusion.

The difference between mean rumen particle size (176 μm) and mean faecal particle size (75 μm for UWS and on average 60 μm for rations based on ammoniated wheat straw) could, at least partly, be attributed to the fact that sieves used for rumen samples had a larger pore size than those used for faecal samples. However, smaller particles have a relatively higher probability of passage than larger (Poppi *et al.*, 1980), which could also explain the smaller mean size for faecal than for rumen particles. Bosch *et al.* (1993b) observed for wilted grass silages fed to dairy cattle mean faecal particle sizes varying from 25-102 μm.

The observation that the mean faecal particle size was smaller for ammonia-treated wheat straw-based diets than for UWS, whereas no difference between rations was found with regard to particle size distribution within the rumen small particle pool, suggests that for the ammoniated wheat straw-based rations k_p of the smallest particles was higher than for UWS.

The rate of passage estimated by chromium mordanted NDF was higher for rations based on ammonia-treated wheat straw than for UWS, but no ration effect on rate of passage of the indigestible NDF fraction was found. The estimates based on Cr-NDF refer to indigestible particles in the rumen with a high functional specific gravity (FSG) and, hence, a relatively high fractional outflow rate (Sutherland, 1987). Indigestible NDF is not physically separated from digestible NDF. Fermentation gases will therefore be entrapped in iNDF containing particles, thus reducing the FSG and consequently the probability of passage. This means that the FSG of particles is related to the D/U ratio. The D-fraction of

the rumen NDF pool was higher for AWS and AWSP than for UWS. Passage rates of identical particles (Cr-NDF) could thus be different between rations, while those of iNDF are similar. Differences in FSG between Cr-NDF and iNDF could also explain why $k_{p\text{-faeces}}$ was significantly higher than $k_{p\text{-iNDF}}$, even if the latter was expressed on basis of the rumen small particle iNDF pool.

The $k_{p\text{-faeces}}$-values in the present experiment were higher than those observed for wilted grass silages in dairy cattle (average 4.5 %/h) reported by Bosch *et al.* (1993b). This indicates that the probability of passage of small particles with a high functional specific gravity is not lower for straw based diets than for diets based on higher quality roughages. Probability of passage is more determined by FSG than by particle size (Kaske *et al.*, 1992). Effective passage rates will therefore be more related to the average FSG in the rumen or in the rumen SP pool than to the k_p determined by Cr-NDF.

Passage is also related to the rumen pool size of particles that can potentially leave the rumen. Rumen DM pool sizes (g/kg liveweight) in the present experiment were 16.5, 18.2 and 16.7 for UWS, AWS and AWSP, respectively (SEM 0.53, ns) in line with the average value of 17.5 reported by Bosch *et al.* (1993b) for wilted grass silages fed during late lactation to dairy cattle. Bosch *et al.* (1993b) observed a slightly higher proportion of small particles in the rumen DM pool (range 74-81 %) than found in the present experiment. As indicated earlier, the $k_{p\text{-faeces}}$ in the present experiment was, however, higher. Effective passage of small particles (k_p times SP-pool) from the rumen in the present experiment could therefore be approximately similar to that in the experiments of Bosch *et al.* (1993b).

The proportions of small particles in the rumen did not differ significantly between rations, while also rumination times per kg NDF ingested were approximately similar for all rations. This suggests, that the rate of particle size reduction during rumination did not differ between rations. However, Doyle (1983) reported, that the resistance to comminution reduces due to alkali treatment as indicated by the fact, that energy consumption in milling was lower for alkali treated rice straw than for untreated rice straw. There were some weak indications of a reduced resistance to particle size comminution after ammonia treatment in the present experiment: UWS compared with AWS and AWSP had a lower rate of comminution ($P > 0.05$) and a higher (also insignificant) LP fraction immediately upon ingestion and a (again insignificantly) longer eating time per kg NDF ingested. The rates of comminution observed in the present experiment were in line with those reported by Bosch *et al.* (1993b) for wilted grass silages (average 5.7 %/h).

The daily time spent ruminating for the straws in the present experiment was approximately similar to that for grass silages fed to dairy cattle reported by Bosch *et al.* (1993b). These findings support the suggestion of Welch (1982) that the maximum duration of rumination is approximately 9-10 hours. Total chewing time per kg NDFI in the experiments of Bosch *et al.* (1993b) was on average 132 minutes, not much different from the average value observed in the present experiment. However, average total chewing time per kg DOMI was 99 minutes in the experiments of Bosch *et al.* (1993a,1993b) and in the present experiment 182 minutes. This indicates, that the energy expenditure in chewing as

proportion of digestible energy intake was higher for straw-based diets than for the silages in the experiments of Bosch *et al.* (1993a,1993b), thus causing a lower efficiency of metabolizable energy utilization.

Maximum rumen fill (Bosch *et al.*, 1993b), time required for mastication (Welch, 1982), protein/energy ratio in digestion products (Egan, 1977), energy demand in relation to physiological status (Weston, 1982) or minimized oxygen consumption per unit net energy ingested (Tolkamp & Ketelaars, 1992) are mentioned as determinants of intake of low quality roughages. These factors are, however, often associated and invalidation of any theory of intake regulation is therefore difficult.

In the present experiment rumen DM pools did not differ between rations, which could indicate, that intake was limited by rumen fill. However, the total rumen pool size decreased during periods between morning and evening feeding, despite the animals had access to straw during this period. Straw intake during this period was, therefore, not limited by potential rumen fill, but it could be that the maximum rumen fill was not maintained between feedings because eating time was restricted by time required for rumination.

Small intestinal protein availability was probably different between rations. Microbial protein synthesis in the rumen is related to energy availability in the rumen, which was higher for AWS and AWSP than for UWS and the potato protein of a low rumen degradability in AWSP increased the dietary protein availability in the small intestine. Therefore, the observed differences with regard to DOMI between treatments could be related, potentially, to differences in small intestinal protein availability.

The fact that DOMI differed between rations indicates that the energy demand of the growing steers was not limiting energy intake, at least for rations UWS and AWS.

Oxygen consumption per unit net energy intake is related to metabolizable energy losses in digestion and metabolic processes and in rumination and eating activity. The metabolizable energy losses in eating and rumination activity as indicated by the time spent ruminating per kg DOMI were lower (though insignificantly) for AWS and AWSP than for UWS and were lower for the silages in the experiments of Bosch *et al.* (1993b) than for the straws in the present experiment. In addition, total metabolizable energy losses increase with decreasing quality of the feed (Agricultural Research Council, 1980). Intake differences within the present experiment and between the present experiment and those reported by Bosch *et al.* (1993a, 1993b) could therefore also be related to the different levels of net energy intake, for which oxygen consumption per unit net energy intake was minimal.

In conclusion, ammonia treatment increased intake and digestion of wheat straw by steers, while potato protein supplementation to ammoniated wheat straw increased total DOMI, but had no effect on OMI and OMD of ammoniated wheat straw. The increased rumen turnover was associated with a higher rumen degradation for ammoniated wheat straw, mainly caused by an increased D fraction. Comparison with wilted grass silages, as an example of a better quality roughage with a higher NDFI and NDF digestion, showed that

differences between silage and straw based diets with regard to rate of comminution and passage characteristics were small. However, the straws offered in the present experiment had a lower k_d than grass silages. In addition, untreated wheat straw had a lower D fraction of NDF than wilted grass silage.

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4.4 Validation of rate constants of comminution, degradation and passage by model estimates derived from rumen evacuation studies in steers

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Abstract

A conceptual rumen model was designed to estimate from a rumen evacuation study with steers fed untreated or ammonia treated wheat straw the rate of degradation (k_d) of large and small rumen particles, the rate of passage of rumen neutral detergent fibre (NDF) particles (k_p) and the rate at which the rumen large particle pool was comminuted to small particles by rumination (k_c). These rate constants were compared with those determined in the same experiment by conventional methods *i.e.* dacron bag incubations for estimation of k_d, faecal excretion of chromium mordanted NDF for estimation of k_p and determination of the decrease of the rumen truly undegradable large particle pool when feed was withheld for estimation of k_c. Model estimates of k_d of the rumen small particle pool were significantly higher than those determined by dacron bag analysis. Estimates of k_d of the rumen large particle pool by the model or from dacron bag analysis were not significantly different. The model estimates of k_p of rumen undegradable small particles were significantly lower than those derived from chromium mordanted NDF. The estimate of k_c by the model was significantly different from the estimates based on the decrease in the rumen large particle pool during fasting for two of the three rations in the experiment. As a result of overestimation of k_p and underestimation of k_d of the rumen small particle pool by the conventional methods of determination, rumen turnover of NDF was overestimated and rumen degradation of NDF underestimated when these rate constants were applied in the model. An analysis of the sensitivity of model predictions of intake and rumen degradation of NDF for changes in model parameters showed that intake was most sensitive to changes of the rumen pool size and of the potentially degradable fraction of the straw, and that rumen degradation was most sensitive to changes of the potentially degradable fraction of the straw and the k_d. Intake of digestible NDF was most sensitive to changes of the potentially degradable fraction of the straw, the k_d and the rumen NDF pool size. Changes of k_c and k_p had only marginal effects on model estimates of intake of digestible NDF.

Keywords: rumen modelling, rate of comminution, rate of passage, rate of degradation, sensitivity elasticity.

Introduction

In a steady-state situation in the rumen the amount of feed entering is equal to the amount leaving the rumen, either by fermentative degradation and absorption or by passage as undegraded feed particles to the lower gut. Feed turnover in the rumen can be described by a model incorporating the rumen pool size and the rates of degradation and passage. Such a model can have the objective to integrate parameters of degradation and passage for feed evaluation, to provide better knowledge of the relation between processes in the rumen or to evaluate methods of estimation of rate constants (Poppi et al., 1981; Aitchisson et al., 1986; Mertens, 1987; Sauvent & Ramangasoavina, 1991).

In this paper a model is described with the principal aim to estimate rate constants of comminution (k_c), fermentative degradation (k_d) and passage (k_p) from rumen evacuation data. These estimates were compared with those obtained from conventional methods to evaluate the quantitative and relative values of these estimates. A second objective of the model was to identify factors limiting rumen turnover and rumen degradation.

The model

Adapted from the model as described by Poppi et al. (1981), a conceptual description of the various processes in the rumen was designed as illustrated in Figure 1. The model given applies to neutral detergent fibre (NDF), which has no water soluble part. The rumen pool is divided into 4 sub-pools: potentially degradable and truly undegradable large particle pools (respectively LPD and LPU) and potentially degradable and truly undegradable small particle pools (respectively SPD and SPU). Large particles were defined as particles retained on a 1.25 mm sieve, which exhibit a low probability of leaving the rumen (Kennedy and Poppi, 1984). Small particles pass through a 1.25 mm sieve.

The turnover of the large particle pool is a function of the rate of degradation of LPD ($k_{d(LP)}$, fraction per day) and the rate of comminution (k_c, fraction per day) of the whole rumen LP pool. The following equations describe the steady state situation of the rumen LP pool:

(1) $LPU_i = LPU_r * k_c$
(2) $LPD_i = LPD_r * (k_c + k_{d(LP)})$
(i = input; r = rumen pool).

The turnover of the small particle pool is a function of the rate of passage of SPU ($k_{p(SPU)}$, fraction per day), the rate of passage of SPD ($k_{p(SPD)}$, fraction per day) and the rate of degradation of SPD ($k_{d(SP)}$, fraction per day) and are described by the following equations:

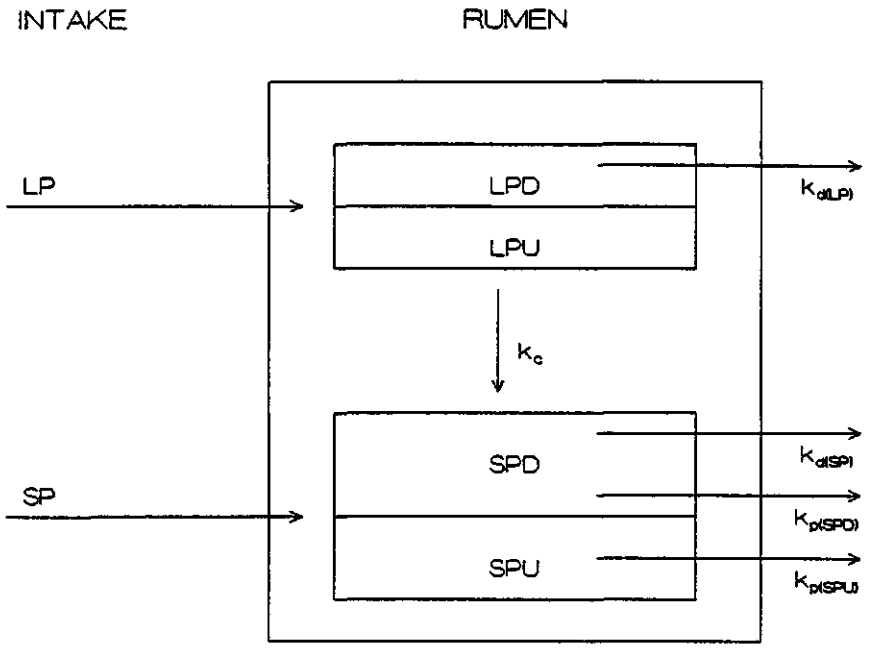

Figure 1. The conceptual rumen model

(3) $SPU_i + LPU_i = SPU_r * k_{p(SPU)}$
(4) $SPD_i + LPD_r * k_c = SPD_r * (k_{d(SP)} + k_{p(SPD)})$

Intake (I) and rumen degradability (D) of NDF can be described by the following formulas:

(5) $NDFI = k_{d(LP)} * LPD_r + (k_{d(SP)} + k_{p(SPD)}) * SPD_r + k_{p(SPU)} * SPU_r$
(6) $NDFD = (k_{d(LP)} * LPD_r + k_{d(SP)} * SPD_r) / NDFI$

Equation (4) contains two unknown parameters: $k_{d(SP)}$ and $k_{p(SPD)}$. A solution for $k_{d(SP)}$ can be derived from equation (6) by using the actually measured values for NDFD and NDFI. NDFD in formula (6) only refers to rumen degradation and does not include hind gut fermentation. The actually recorded in vivo NDFD was converted to rumen NDFD by

accepting a partial digestion (fraction of whole tract digestion) of 0.85 in the rumen (Aitchisson et al., 1986).

One assumption on which the model was based was that processes of degradation, passage and comminution obey first order kinetics. There are no apparent reasons to reject first order kinetics for degradation in dacron bags (Robinson et al., 1986) and for excretion of markers from the rumen (Faichney, 1975). Another assumption was, that the rumen is in a steady-state condition. Diurnal variation in rumen pool size (Oosting et al, 1993) and in rate of passage of the fluid phase (Gasa et al., 1991) and of the particulate phase (Girard, 1990) have been reported. Diurnal variation in rate of degradation is also quite likely associated with fluctuations of the rumen pH. Diurnal variations will, however, be greater than day to day variation. It seems therefore justified, that the average daily rumen pool size and the rates of comminution, passage and degradation are regarded as steady state parameters.

The model also assumes differences in k_p between SPD and SPU. Differences between rates of passage of potentially degradable and truly undegradable NDF were observed by Tamminga et al. (1989). They reported rates of passage for the potentially degradable and truly undegradable rumen NDF pool of 1.3 and 3.3 %/h, respectively.

The model was applied to the data of a rumen evacuation study reported by Oosting et al. (1993) in which untreated wheat straw (UWS), ammonia treated wheat straw (AWS) and ammonia treated wheat straw supplemented with potato protein of a relatively low rumen degradability (AWSP) were fed to steers in a 3 x 3 Latin square design. All rations were supplemented with a sugar beet pulp supplement at the level of 18.2 g DM/$kg^{0.75}$/day. In this experiment the average size of the rumen pool was determined by rumen evacuation on three consecutive days, one day at 09.30 h, one day at 13.30 h and one day at 17.30 h. The conventional methods of estimation of the rate constants of comminution referred to in the present paper were:

- dacron bag incubations of large and small rumen particles for estimation of the rate of degradation (k_d) of LPD and SPD,
- faecal excretion of chromium mordanted NDF for estimation of the rate of passage (k_p) of (SPD + SPU),
- the decrease of the rumen large particle NDF pool in fasting animals with a correction for the rate of degradation of LPD for estimation of the rate constant of comminution (k_c).

Table 1. Parameters associated with intake, rumen pool size and whole-tract digestion of NDF from the experiment of Oosting et al. (1993).

	UWS	AWS	AWSP
NDFI (g/day)			
- *straw*	4669	5333	5417
- *supplement*	584	568	605
D-fraction			
- *straw*	0.67	0.78	0.78
- *supplement*	0.94	0.94	0.92
LP fraction of ingested straw:	0.56	0.46	0.46
Rumen NDF pool (g)	5793	6096	5720
LP fraction of pool	0.31	0.33	0.31
D fraction			
- *LP*	0.59	0.69	0.71
- *SP*	0.55	0.62	0.59
Whole-tract NDFD (g/kg)	525	595	617

Relevant data from the rumen evacuation study on NDF intake, rumen NDF pool and whole tract NDF digestibility are given in Table 1. Model estimates of rate constants were based on measurements per animal per period. Only for the large particle fraction of the ingested straw one average value for UWS as well as for AWS was taken, since only two estimates per straw were available. The D fraction of straw NDF is the D fraction of the non-soluble part. Approximately 16 % of NDF disappeared after rumen incubation of 0.5 h. However, this apparent solubility should be attributed to escape of small particles. It was therefore assumed that the D fraction of this apparently soluble part of straw NDF was equal to the D fraction of the insoluble part. The D fraction of the NDF of the supplement was equal to 100 minus the U fraction of the organic matter, since this U fraction of NDF could not be determined. For calculation of the LPU, LPD, SPU and SPD fractions of the ingested NDF it was accepted that the D fractions of ingested LP and SP were similar and that the supplement consisted completely of SP. Estimation of the D fractions of rumen LP and SP was done by dacron bag incubation of isolated LP and SP. Not all small particles were included in the isolated SP fraction, since this fraction was isolated by sieving over dacron cloth with a pore size of 0.041 mm. It was assumed, that particles passing through the dacron cloth had the same D fraction as the isolated SP.

To test the sensitivity of model predictions of NDFI, NDFD and DNDFI (digestible NDF intake) for changes of one of the model parameters the following procedure was followed: The required relative increase of one of the model parameters to get a relative increase of straw NDFI of 10 % was calculated. Except the one parameter, that was increased and consequently the proportions of LPU, LPD, SPU and SPD in the rumen all other parameters were kept constant. Also the intake of the supplement was kept constant.

NDFD and DNDFI were calculated for the new situation. The sensitivity of the model predictions of NDFI of the whole ration, NDFD and DNDFI for change of one of the model parameters was expressed by the sensitivity elasticity, which was defined as:

(7) $(\Delta y/y)/(\Delta x/x)$
(y = NDFI, NDFD or DNDFI, x = model parameter, Δ = increase (value after change minus basal value).

Statistical analysis was done by the program DBSTAT (Brouwer, 1989). To test the effect of rations the data were analysed according to the model

$$Y_{ijkl} = \text{mean} + \text{Period}_i + \text{Animal}_j + \text{Ration}_k + \text{error}_{ijkl}.$$

Total degrees of freedom (d.f.) was 9 and each of the factors and the error had d.f. = 2. Significance of differences between rations was tested by Student's t-test.

The difference between model predictions of rate constants and estimates derived from conventional methods was calculated and subjected to the above described procedure to test the difference between methods of estimation of rate constants.

Testing of homogenity of variance between methods of estimation of rate constants was done by analysis of the absolute value of the residuals (observed minus predicted value) by the model:

$$Y_{ij} = \text{mean} + \text{method}_i + \text{error}_{ij}.$$

Total d.f. = 18, d.f. error = 16. This procedure was described by Levene (1960).

Results.

Model predictions for k_c, $k_{d(LP)}$, $k_{p(SPU)}$, $k_{d(SP)}$ and $k_{p(SPD)}$ and estimates of the same parameters (except $k_{p(SPD)}$) from conventional methods are given in Table 2. For model estimates of rate constants no significant differences were observed between rations. The conventionally derived estimates of rate constants did also not differ significantly between rations for $k_{d(LP)}$ and k_c, but $k_{p(SPU)}$ was significantly higher for AWS and AWSP than for UWS and $k_{d(SP)}$ was significantly higher for AWSP than for AWS and UWS.

The difference in k_c between methods of estimation was significantly different from zero for AWS and AWSP and for the mean over rations, indicating that, with the exception of UWS, the conventional method resulted in significantly higher estimates of k_c than did the model. The differences between model and conventionally derived estimates within rations

did not differ significantly from zero for all other rate constants, but the difference of means over rations differed significantly from zero for $k_{p(SPU)}$ and $k_{d(SP)}$.

The average absolute value of residuals was significantly higher for model than for conventionally derived estimates of $k_{d(LP)}$, $k_{p(SPU)}$ and $k_{d(SP)}$. No significant difference was found between the average absolute value of residuals between model and conventionally derived estimates of k_c.

No significant correlations were found between model and conventionally derived estimates for any rate constant. Model estimates were not significantly correlated, except $k_{p(SPU)}$ and $k_{p(SPD)}$ ($r = 0.80$, $P < 0.01$) and $k_{p(SPD)}$ and $k_{d(SP)}$ ($r = 0.69$, $P < 0.05$).

The model estimates of $k_{d(SP)}$ were higher than those of $k_{d(LP)}$, but the difference between the two was not significantly different from zero ($P < 0.10$). The difference between model estimates of $k_{p(SPU)}$ and $k_{p(SPD)}$ tended ($P < 0.10$) to be higher than zero.

NDFI, NDFD and DNDFI were predicted by the model from rumen pool sizes and conventionally derived estimates of rate constants, with the assumption, that $k_{p(SPU)}$ was equal to $k_{p(SPD)}$. A considerable overestimation of NDFI was found. Predicted NDFI was 1.20, 1.25 and 1.26 times the observed NDFI for UWS, AWS and AWSP, respectively. NDFD was underestimated. Predicted NDFD was 0.45, 0.51 and 0.53 times the observed NDFD, calculated as 0.85 times the whole tract NDF digestion for UWS, AWS and AWSP, respectively.

Table 2. Model predictions and estimates based on conventional methods of rate constants.

	UWS	AWS	AWSP	SEM	mean over rations (SEM)		Mean absolute value of residuals‖
k_c (%/h)							
- *model*	5.0	3.6	4.4	0.48	4.3	(0.28)	0.37
- *conventional*	4.1	6.3	7.3	0.59	5.9	(0.34)	0.40
- *difference*§	$0.9^{a,ns}$	$-2.7^{b,**}$	$-2.9^{b,**}$	0.25	-1.6^{**}	(0.25)	
$k_{d(LP)}$ (%/h)							
- *model*	2.2	2.1	1.9	0.81	2.1	(0.47)	0.55^{d}
- *conventional*	1.5	2.2	2.0	0.19	1.9	(0.11)	0.14^{c}
- *difference*§	0.6^{ns}	-0.1^{ns}	-0.1^{ns}	0.99	0.2^{ns}	(0.57)	
$k_{p(SPU)}$ (%/h)							
- *model*	3.7	3.6	3.3	0.84	3.5	(0.49)	0.62^{d}
- *conventional*	5.4^{a}	6.1^{b}	6.3^{b}	0.12	5.9	(0.07)	0.10^{c}
- *difference*§	-1.7^{ns}	-2.5^{ns}	-3.0^{ns}	0.92	-2.4^{*}	(0.54)	
$k_{d(SP)}$ (%/h)							
- *model*	3.6	4.1	4.7	1.00	4.1	(0.57)	0.74^{d}
- *conventional*	1.8^{a}	1.8^{a}	2.0^{b}	0.02	1.9	(0.01)	0.01^{c}
- *difference*§	1.8^{ns}	2.3^{ns}	2.6^{ns}	0.93	2.2^{*}	(0.52)	
$k_{p(SPD)}$ (%/h)							
- *model*	2.5	3.0	3.0	0.53	2.8	(0.30)	0.37

Different superscripts a and b indicate significant differences ($P < 0.05$) between rations).
§ Superscripts ns ($P > 0.05$), * ($P < 0.05$), ** ($P < 0.01$) indicate significance of difference from zero.
‖ Different superscripts c and d indicate significant differences ($P < 0.05$) between model and conventional method.

Figures 2, 3 and 4 give the sensitivity elasticity of NDFI, NDFD and DNDFI to changes in model parameters for the three rations. Model parameters for which the sensitivity elasticity was calculated were:

- total rumen NDF pool size
- k_c
- SP_i, which is the SP fraction of straw NDFI
- k_p, which is the k_p of SPU and SPD. It was assumed, that the relative increases of $k_{p(SPU)}$ and $k_{p(SPD)}$ were equal.
- $k_{d(LP)}$
- $k_{d(SP)}$
- k_d, assuming the same relative increase for $k_{d(LP)}$ and $k_{d(SP)}$.
- D_i, which is the D fraction of straw NDF.

Model predictions of NDFI were most sensitive to changes in rumen pool and D_i (Figure 2). The rumen pool had to increase by 10.4 % and the D_i for AWS and AWSP on average by 13.9 % to get a 10 % higher NDFI, while for UWS, D_i had to increase by 24.6 %. The model prediction of NDFI was least sensitive to k_c, $k_{d(LP)}$ and $k_{d(SP)}$. The required relative increases of these parameters to get a 10 % higher NDFI were on average 71.4, 109.6 and 52.1 %, respectively, with only small differences between rations. The sensitivity of model prediction of NDFI was intermediately sensitive to SP_i, k_d and k_p. On average these parameters had to increase by 37.0, 32.1 and 24.9 %, respectively, to get a 10 % relative increase of NDFI, with only small differences between rations.

Model predictions of NDFD were most sensitive to an increased D_i (Figure 3). Increases of rumen pool, k_c, SP_i, and $k_{d(LP)}$ hardly affected NDFD. The sensitivity elasticity was negative for k_p and positive for $k_{d(SP)}$ and k_d.

Model predictions of DNDFI were most sensitive to D_i. Increases of rumen pool size, $k_{d(SP)}$ and k_d also had considerable effects on relative increase of DNDFI, but k_c, SP_i, k_p and $k_{d(LP)}$ hardly affected DNDFI (Figure 4). In general the experimental diets behaved similarly.

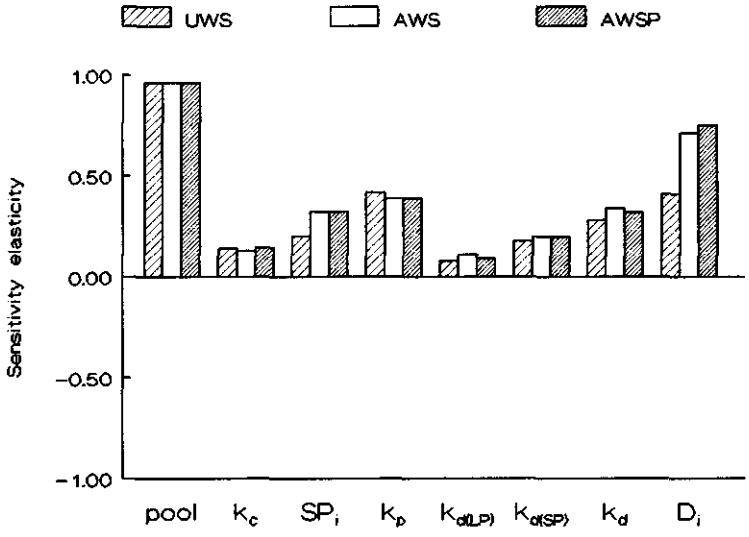

Figure 2. Sensitivity elasticity of NDFI for model parameters

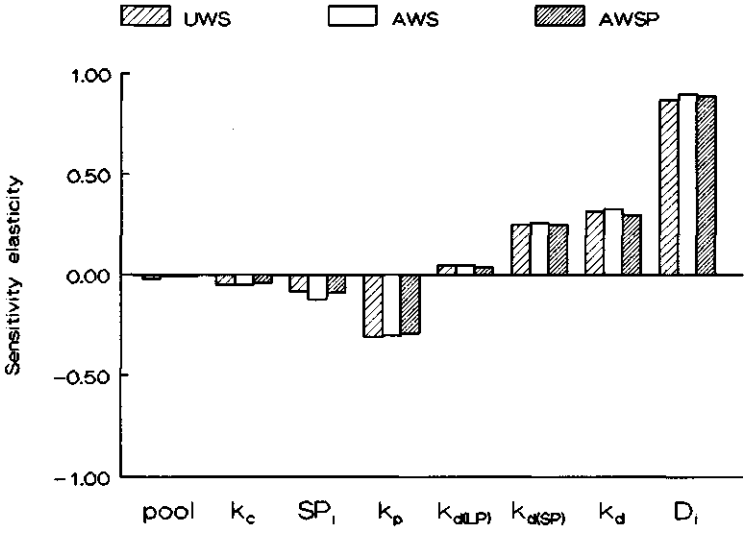

Figure 3. Sensitivity of NDFD for model parameters

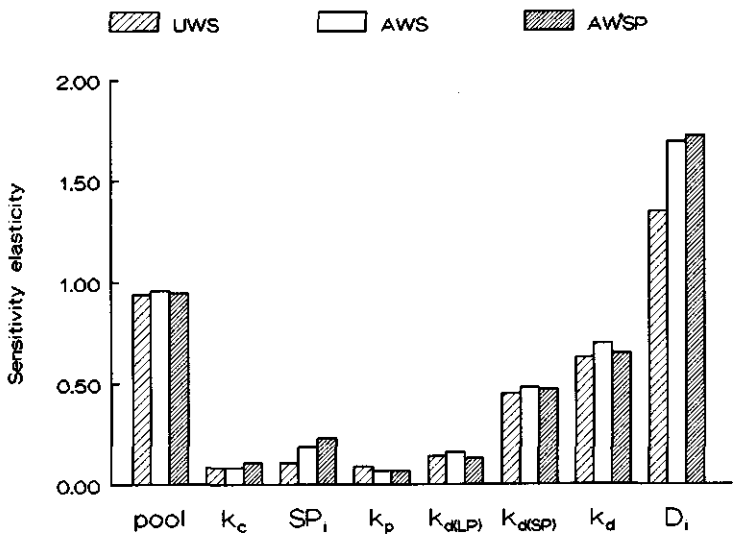

Figure 4. Sensitivity of DNDFI for model parameters

Discussion

The model described in the present paper served as a tool to estimate rate constants from a rumen evacuation study and to identify limiting factors for rumen turnover and digestion. The residuals of model estimates of rate constants were, except for k_c, significantly higher than those for conventionally derived estimates. This is a result of the fact, that all errors associated with estimation of intake and rumen pool parameters are reflected in the model estimates of rate constants. Improved accuracy of model predictions could be achieved by increasing the accuracy of estimates of intake and rumen pool parameters by repeated measurements per animal. The weight of total rumen contents varied within a day in the rumen evacuation study (Oosting et al, 1993), which means that average daily size and composition of the rumen pool should be derived from more frequent measurements than the three applied in the experiment reported by Oosting et al. (1993).

Although the conventional methods gave more accurate estimates of rate constants than the model, they are probably not better, because they give a biased description of actual processes in the rumen. Chromium mordanted NDF consists of small indigestible particle with a high functional specific gravity, probably higher than that of the SPU fraction in the rumen. Since rate of passage is positively correlated with functional specific gravity (Sutherland, 1987), chromium mordanted NDF will presumably have a higher rate of passage than SPU. In the present study, model estimates of the rate of passage of SPU were

significantly lower than the estimates based on chromium mordanted NDF. Aitchisson et al. (1986) also observed lower model estimates of k_p than when estimated by use of chromium mordanted NDF. The rate of passage of SPD is even more overestimated by chromium mordanted NDF. Tamminga et al. (1989) reported a lower k_p for potentially degradable NDF than for truly undegradable NDF and in the present study the model predictions of rate of passage of SPD tended to be lower than those of SPU. It can thus be concluded that estimation of the rate of passage by chromium mordanted NDF does not give reliable quantitative information about passage processes in the rumen. At best, some qualitative information about rumen passage is given by k_p derived from chromium mordanted NDF, although estimates based on chromium mordant and model predictions of k_p were not significantly correlated in the present study. This latter aspect, however, could also be attributed to the relatively low between treatment variation and high standard errors of estimates, especially for the model predictions. The model calculations showed that rate of passage of SPD was positively correlated with rate of passage of SPU. If such a positive correlation would exist for all rumen pools that can potentially leave the rumen, it would mean that chromium mordanted NDF could give relative information about k_p of rumen fractions.

The k_d of small particle NDF was significantly lower when estimated from dacron bag incubations than from the model, while the rate of degradation of large particle NDF did not differ significantly between methods of estimation. Aitchisson et al. (1986) also found higher model than conventionally derived estimates for k_d. Although model and dacron bag based estimates of k_d were not significantly correlated, the effect of rations was too small and the errors associated with estimation of k_d too large to draw meaningful conclusions about the relative value of dacron bag based estimates for k_d. The fact, however, that $k_{d(LP)}$ was not differing between methods of estimation and that $k_{d(SP)}$ was higher for model estimates than for conventionally derived estimates, indicates that the relative value of k_d estimated from dacron bag incubations should be regarded cautiously.

Use of the model as described in the present paper resulted in estimates of the rate of comminution of large particles that were lower for rations AWS and AWSP than the estimates obtained from the decrease of the large particle pool in animals that had no access to feed. The model estimates of k_c were based on rumen pool sizes when the steers had free access to feed. Animals without access to feed probably spent more time ruminating, which could explain why the convential estimates of k_c were higher than the model predictions for AWS and AWSP.

The sensitivity analysis as applied gives information about factors limiting rumen turnover and rumen degradation. A high sensitivity elasticity for a parameter means that the parameter is more limiting than a parameter with a low sensitivity elasticity. Sensitivity analysis of model predictions of NDFI showed that NDFI was most limited by the rumen pool size and the potential degradability of the straw NDF. The size of the potential degradable fraction was less limiting for UWS than for AWS and AWSP. The k_c and the $k_{d(LP)}$ did hardly affect NDFI.

The sensitivity of NDFD was higher for an increase of the D fraction of the straw than for an increase of the k_d. The sensitivity elasticity of NDFD was negative for k_p, which could be attributed to a shorter retention time. The rumen pool size, k_c and small particle fraction of the ingested feed hardly affected NDFD.

In turn, model predictions of DNDFI were most sensitive to an increase of the D fraction of the straw, the rumen pool size of NDF and the k_d. Increases of k_c, the fraction of small particles in the ingested straw and k_p did not affect DNDFI.

Although it should be kept in mind that the model is probably an oversimplification of the processes in the rumen, the sensitivity analysis showed that steers fed either untreated or ammoniated wheat straw could not increase their intake of digestible NDF substantially by ruminating or chewing more or more intensively or by increasing the rate of passage. However, rumination has likely more functions than conversion of LP into SP. Rumination could play an essential role in increasing the functional specific gravity of particles.

The sensitivity elasticity of model predictions of DNDFI for k_p and k_c were also calculated taking a 10 % higher NDFI than the observed as the basal level. This showed that the sensitivity elasticity of DNDFI for k_c and k_p was still low at a high intake level (on average 0.15 and 0.16, respectively), but higher than at a low intake level (on average 0.09 and 0.08, respectively), if the higher intake was a result of an increased k_d. If the higher intake was a result of an increased D_i, the sensitivity of the model predictions of DNDFI for k_c and k_p were similar for the low and high intake level. The low importance of the extent of D_i on sensitivity elasticity of DNDFI for k_c and k_p can also be illustrated by the difference in sensitivity elasticity between AWS, AWSP and UWS at the observed feed intake level; Ammoniation of wheat straw increased the potentially degradable fraction, without affecting the sensitivity of DNDFI for changes of k_p and k_c. However, the sensitivity elasticity of DNDFI for increase of the small particle fraction in the ingested feed was almost doubled from 0.11 to 0.21 after ammoniation of wheat straw, but this remained still low if compared to the sensitivity elasticity of DNDFI for other factors.

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CHAPTER 5. GENERAL DISCUSSION

Chapter 5. General discussion

In many tropical countries and especially in South-East Asia, production of ruminant livestock in terms of milk, meat, draught power and dung, becomes more and more dependent on fibrous crop-residues. Grazing lands and lands used for forage production are increasingly utilized for cultivation of food-crops required to feed the steadily growing human population. In turn, the demand for livestock production increases as a result of this growing population and the increasing *per capita* income. In addition, in many tropical countries the majority of the population finds employment in agriculture. Agriculture should be, therefore, labour-intensive to absorb the growing labour force in rural areas. Under these circumstances, livestock production from small-scale farms making use of the locally available fibrous crop-residues seems the most preferable system in respect of social-economic sustainability.

Sustainability of agricultural production systems in respect of preservation of the natural environment for future generations should be a matter of great concern. Natural resources, like soil- and water-quality and availability of water are threatened by human activities, like industrialization, deforestation and agriculture in many tropical countries. Deforestation may result in climatic changes and erosion and the low level of fertilization applied in low-input agricultural systems may cause depletion of nutrient levels in soils. Future generations could be faced therefore with the fact that they have to produce food from a diminishing area of land suitable for agricultural production. Obviously, reduction of the population growth rate in rural areas could contribute most to sustainability of agricultural systems in the tropics, but is not easy to achieve. Ruminant livestock may play an important role in sustainable agricultural production systems, because they can produce valuable products, including dung, from fibrous crop-residues and products from (leguminous) trees planted in reforestation programs.

The quality of fibrous crop-residues is often too low to achieve livestock output. Intake and digestion are relatively low. Treatments to increase the nutritive value and supplementation with feeds of a higher quality are required to get production in terms of meat and milk. Knowledge of factors limiting intake and/or digestion of fibrous crop-residues could contribute to formulation of treatments and/or supplementation strategies to increase the production from rations based on these fibrous crop-residues.

In the experiments reported in the present thesis, wheat straw (as a model for fibrous crop-residues in general) was treated with ammonia and/or supplemented with energy and protein. The effects of these experimental treatments on voluntary energy intake and parameters associated with rumen degradation, rumen microbial protein synthesis and small intestinal protein availability were investigated. In the following sections of this chapter the effects of the various experimental treatments on these parameters are discussed. Part of the experiments were conducted with sheep, while crop-residues are mainly fed to cattle. Therefore results obtained with sheep and cattle will be compared.

Ammonia treatment of wheat straw

In all experiments reported in this thesis, ammonia treatment of wheat straw significantly increased voluntary intake and digestion of organic matter. The increased quantity of straw ingested as a result of ammonia treatment is additionally processed in the rumen. In two experiments with sheep (Chapters 3.1 and 4.1), it was observed that the increased disappearance of ingested cell walls from the rumen could for 85 % (average across both experiments) be attributed to an increased rumen degradation and only for 15 % to an increased passage of undegraded feed particles from the rumen. The rate of degradation of either ingested feed or the rumen cell wall pool did not differ significantly between untreated and ammonia-treated wheat straw. The increased rumen degradation as a result of ammonia treatment can therefore largely be attributed to a higher potentially degradable fraction in ingested feed and, as a consequence, in the rumen cell wall pool.

The fractional rate of passage (k_p) of the marker Cr-NDF, estimated from the logarithmic decline of marker concentration in faeces, did not differ between untreated and ammonia-treated wheat straw-based rations in sheep. Contrary to this, in steers (Chapter 4.3) a significant difference was found between both diets for this parameter, but not for the rate of passage of indigestible cell walls from the rumen. The latter gives probably a more reliable estimate of the rate at which passage from the rumen occurs, because it takes also into account differences in functional specific gravity of particles that can potentially leave the rumen. The k_p derived from the faecal excretion pattern of Cr-NDF refers to particles with a relatively high functional specific gravity and probably overestimates therefore the k_p of rumen small particles (see discussion in Chapters 4.3 and 4.4). Also in steers, the increased disappearance of cell walls from the rumen as a result of ammonia treatment could be attributed, therefore, mainly to the increased potential degradability and to a lesser extent to an increased passage.

As illustrated in Chapter 4.4, a model analysis revealed that an increased rate of passage was associated with a higher intake of cell walls, but hardly affected the amount of cell walls degraded in the rumen. For low-quality feeds, that consist almost completely of cell walls, increased passage rates would therefore not result in increased consumption of digestible cell walls, unless a considerable part of the cell walls, passing undegraded from the rumen, would be degraded in the hindgut. In sheep (Chapters 3.1 and 4.1), the contribution of the hindgut to whole-tract digestion was low, however, and was not affected by ammonia treatment.

In sheep, higher rumen fluid volumes were observed in animals fed rations based on ammonia-treated wheat straw compared with those based on untreated wheat straw (Chapter 3.1: $P > 0.05$, Chapter 4.1: $P < 0.05$), indicating that rumen volume as such was not limiting intake of untreated straw-based rations. In steers (Chapter 4.3), however, the rumen volume was significantly lower for the ration based on ammoniated wheat straw than for that based on untreated wheat straw.

Effects of ammonia treatment of straw on rumen pH, volatile fatty acid (VFA)

concentration and proportions of individual VFA's were not observed. Much higher rumen NH_3-N concentrations were found, however, for ammonia-treated straw-based rations than for those based on untreated straw. In most experiments the NH_3-N concentrations found for untreated wheat straw-based rations were below 50 mg/l. Satter and Slyter (1974) found this as the required NH_3-N concentration for maximal microbial protein synthesis *in vitro*. For maximum degradation *in vitro* even higher NH_3-N concentrations were required (Chapter 2.1).

Therefore one could potentially attribute the effect of ammonia treatment on rumen degradation and voluntary intake to an increased rumen supply of nitrogen. However, in the experiment with goats fed untreated wheat straw supplemented with urea (Chapter 2.2) no effect of the NH_3-N concentration in the rumen fluid on *in sacco* rumen degradation was observed. Also in the experiment reported in Chapter 3.1 urea supplementation to untreated wheat straw and the associated increased rumen NH_3-N concentration had no effect on voluntary intake and rumen degradation parameters, while ammonia treatment increased intake and rumen degradation significantly. *In vitro* the substrate-NH_3-N concentration is closely related to the true nitrogen availability for the microbial population, whereas *in vivo* the rumen NH_3-N concentration is a resultant of the rate at which nitrogen is released from the ingested feed, the rate of influx of urea into the rumen, the rate of incorporation into microbial protein and the rates of passage and absorption from the rumen. Therefore a low rumen NH_3-N concentration could also be an indication of a relatively high incorporation or disappearance rate and not necessarily of a limited nitrogen availability only.

Egan and Doyle (1985) and Doyle and Panday (1990) observed an increased small intestinal crude protein availability after supplementation of low-quality roughages with urea. In these experiments no effect of the urea supplementation on rumen degradation was observed, but the increased small-intestinal crude protein supply was presumed the resultant of an increased efficiency of microbial protein synthesis in the rumen. The observations in goats (Chapter 2.2) fed untreated wheat straw supplemented with different levels of urea were in line with the results from these experiments, suggesting that also in this experiment small intestinal crude protein supply increased as a consequence of an increased microbial protein synthesis in the rumen. This would mean that rumen nitrogen availability may limit microbial protein synthesis, while it is not limiting the fermentative activity of the microbes. In the experiment with sheep, reported in Chapters 3.1 and 3.2, it was observed, however, that urea supplementation to untreated straw had no effect on either fermentative degradation or microbial protein synthesis in the rumen. There is no conclusive evidence whether the difference between the experiments with goats and sheep should be attributed to a species difference or to the effect of the additional sugar beet pulp supplement given in the case of this sheep experiment. The conclusion seems justified that, in sheep fed untreated wheat straw supplemented with sugar beet pulp, rumen NH_3-N availability limited neither rumen degradation nor microbial protein synthesis. Consequently, the effect of ammonia treatment could not be attributed to an increased rumen NH_3-N availability.

Energy supplementation

Energy supplementation may negatively affect energy intake from low-quality feeds. Faverdin et al. (1991) observed higher substitution rates (SR, reduction in roughage intake/unit supplement offered) for roughages with a higher than for those with a lower quality. Doyle et al. (1986) reported a higher SR for ammonia-treated rice straw than for untreated rice straw. At low levels of energy supplementation, substitution of roughage by supplement intake may be absent (Doyle et al., 1986) or even negative. Silva et al. (1989) observed positive effects of low supplementation levels of sugar beet pulp on intake and digestion of untreated barley straw, probably as a result of supplying additional energy for microbial degradation in the rumen.

The relationships between *ad libitum* digestible organic matter intake (DOMI) from straw, whole ration and DOMI from the energy supplement are given in Figure 1. From the various experiments presented in this thesis, treatment means per experiment for rations UWS (untreated wheat straw plus energy supplement) and AWS (ammonia treated wheat straw plus energy supplement) are given in this figure. Data for rations supplemented with protein or urea were not included. Results from an experiment reported by Oosting and Van Bruchem (1991), in which intake and digestion of untreated and ammonia-treated wheat straw in goats, sheep and cattle were compared are also given in Figure 1. These animals received a concentrate supplement at a level of 16-18 g organic matter (OM) per $kg^{0.75}$/day. Results for sheep and goats were combined.

The regression lines given suggest linear relationships between $DOMI_{straw}$ and $DOMI_{supplement}$. However, Faverdin et al. (1991) observed a curvilinear relationship between OMI from roughages and energy supplementation level, indicating that the SR increases with the level of energy supplementation. At low energy supplementation levels (below 10 g $DOMI_{supplement}/kg^{0.75}$/day) SR's may be even negative (Silva et al., 1989, De Jong and Van Bruchem, 1993). As a result, the SR for UWS in sheep and goats for energy supplementation levels higher than 10-15 g $DOMI/kg^{0.75}$/day was probably underestimated and, consequently, the intercepts for AWS in sheep and goats and for AWS and UWS in cattle must be presumed overestimated.

Table 1 presents the substitution rates for DOMI ($\Delta DOMI_{straw}/\Delta DOMI_{supplement}$) and for OMI (reduction straw OMI/unit OMI of energy supplement). Within species, statistical analysis of ration effects on substitution rates and intercepts was done by the model: $Y = \text{intercept} + \text{Ration}_i + (b + \Delta b_i) * X$, with b as the overall and Δb_i as the difference between the overall and within ration regression coefficient. Y represents either DOMI or OMI of straw and X is DOMI or OMI of the energy supplement. SR is equal to $-b$.

In sheep and goats, the SR-values were significantly higher for AWS than for UWS, in line with the observations reported by Doyle et al. (1986) for untreated and ammonia-treated rice straw. However, this was only to a lesser extent observed in cattle.

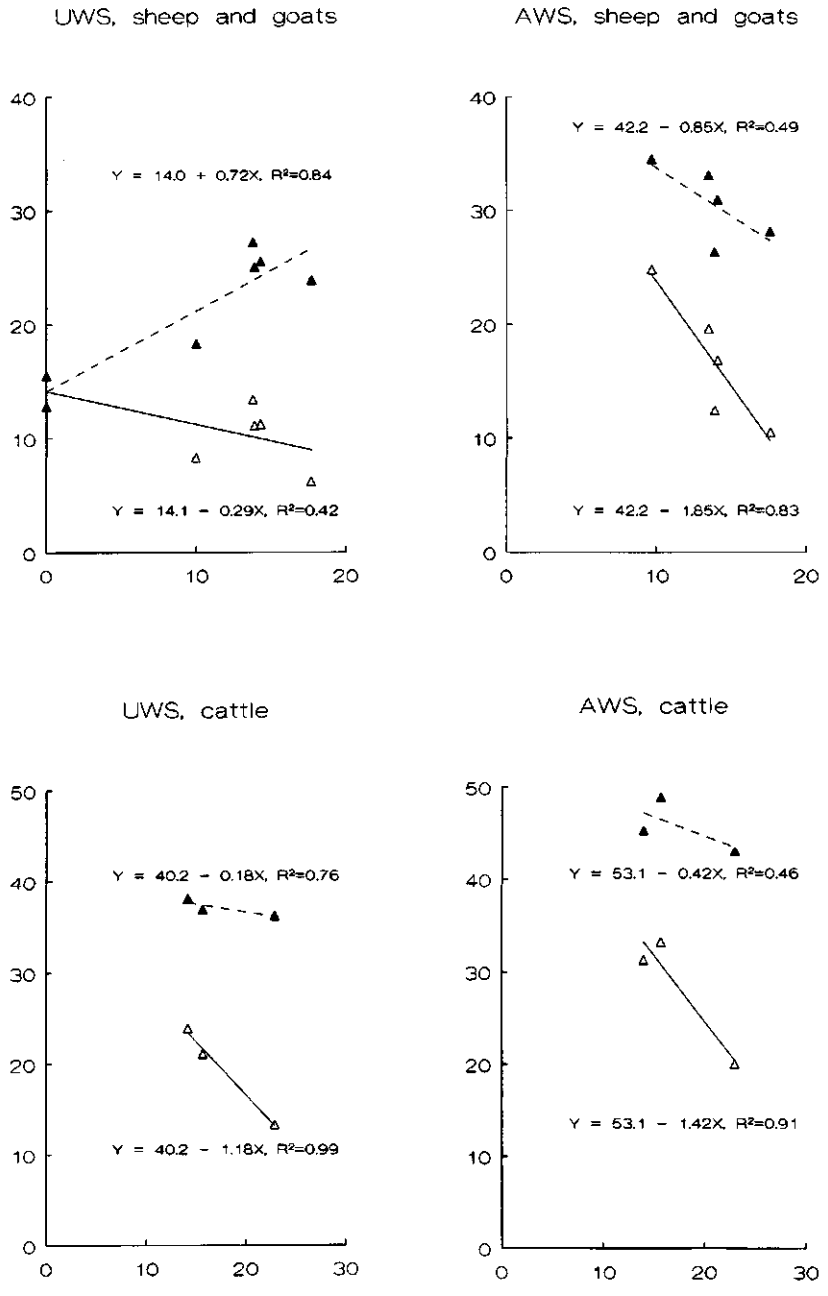

Figure 1. DOMI (Y-axis, g/kg$^{0.75}$/day) in relation to DOMI from energy supplements ($DOMI_{supplement}$, X-axis, g/kg$^{0.75}$/day). (Open symbols: straw; closed symbols: whole ration).

Table 1. Substitution of untreated and ammonia-treated wheat straw by energy supplements (between brackets s.e.m. of estimate).

	Sheep and goats		Cattle	
	UWS (n=7)	AWS (n=5)	UWS (n=3)	AWS (n=3)
Regression $DOMI_{straw}$ on $DOMI_{supplement}$				
- intercept	14.1^a	42.2^b	40.2	53.1
	(1.85)	(6.72)	(6.13)	(5.97)
- SR	0.29^a	1.85^b	1.18	1.42
	(0.156)	(0.480)	(0.340)	(0.331)
Regression OMI_{straw} on $OMI_{supplement}$				
- intercept	31.9^a	49.9^b	63.7	76.5
	(3.50)	(7.00)	(5.10)	(5.04)
- SR	0.42^a	1.00^b	0.94	1.00
	(0.191)	(0.283)	(0.200)	(0.199)

Different superscripts in a row within species indicate significant differences ($P < 0.05$).

Digestion of straw decreased with increasing $DOMI_{supplement}$ (Figure 2). The regression coefficients of individual regression lines did not differ significantly from zero. However, analysis of the pooled data set by a model including ration and species as factors, $DOMI_{supplement}$ as the co-variable and all two-way interactions, showed a significant effect of $DOMI_{supplement}$ (regression coefficient -4.9, s.e. of estimate 1.95) and a significant ration effect (least square means 444 (s.e.m. 13.0) and 537 (s.e.m. 13.4) for UWS and AWS, respectively). Two-way interactions and the species effect were not significant (least square means 514 (s.e.m. 17.6) and 468 (s.e.m. 9.2) for cattle and sheep/goats, respectively).

As indicated in Chapter 3.1 of this thesis, the reduction in rumen degradation as a result of energy supplementation could partly be attributed to a lowered rate of fermentative degradation (k_d) of ingested straw in the rumen. The reduced rumen degradation of straws with increasing supplement intake level could, however, also in part be attributed to the fact that the mean retention time ($100/k_p$) of roughage in the rumen decreased with increasing concentrate intake (Owens and Goetsch, 1986). In the experiment reported in Chapter 3.1 the supplementation of sugar beet pulp was, on average for UWS and AWS, 9.8 g DOMI/$kg^{0.75}$/day and the average k_p (rate of passage of the particulate phase) 2.8 %/h. The k_p in the experiment reported in Chapter 4.1 was on average 3.7 %/h for a supplementation level of 14.2 g DOMI from sugar beet pulp per $kg^{0.75}$/day.

One could attribute the reduced straw intake as a consequence of energy supplementation to a reduction in rumen degradation in line with the hypothesis of physical

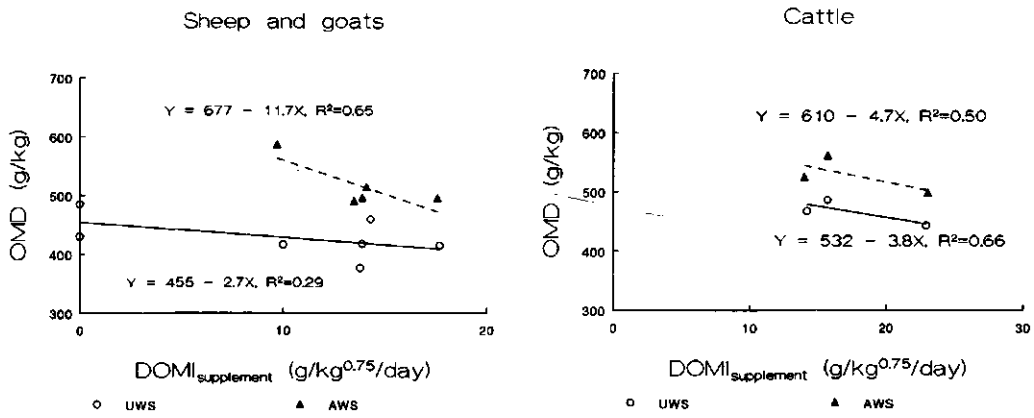

Figure 2. OMD of straw in relation to $DOMI_{supplement}$

regulation of feed intake. However, the decrease in DOMI from whole ration AWS in sheep and goats and UWS and AWS in cattle with increasing $DOMI_{supplement}$ was not significant. This indicates that energy intake from straw was completely substituted by that of the supplement. This was also the case for UWS in sheep and goats at higher energy supplementation levels. The fact that total DOMI stabilized at higher levels of energy supplementation could also indicate that the capacity of animals to utilize energy determined straw intake from diets consisting of straw and energy supplements. The reduced rumen degradation as a result of energy supplementation should then merely be regarded as an associated effect and not as the factor determining straw intake.

Nitrogen supplementation

Nitrogen supplements may act at various levels. Rumen availability of non-protein nitrogen (in the case of urea and protein supplementation) and that of oligo-peptides and branched chain amino acids and minerals (in the case of protein supplementation) may increase by supplementation. If rumen availability of any of these nutrients relative to energy is limited, nitrogen or protein supplementation will increase microbial growth and

fermentative degradation. In addition, the small-intestinal availability of protein may increase owing to nitrogen supplementation as a result of an increased microbial biomass flow and/or an increased passage of undegraded dietary protein from the rumen. Egan (1977), Preston and Leng (1987) and Doyle and Panday (1990) suggested that energy intake from low-quality roughages could be limited by the quantity of protein available for absorption from the small intestine.

Urea supplementation to low-quality roughages increased the small-intestinal crude protein availability and the voluntary digestible energy intake in the experiments of Egan and Doyle (1985) and Doyle and Panday (1990). The similarity between the effect of urea infusion on intake and digestion in these experiments and the one reported in Chapter 2.2 with goats suggests that, also in the latter experiment, the increased DOMI could be attributed to an increased small-intestinal protein supply presumably as a result of an increased efficiency of microbial protein synthesis in the rumen. As indicated in Chapters 3.1 and 3.2, effects of urea supplementation to untreated wheat straw on efficiency of microbial protein synthesis could not be found in an experiment with sheep. The absence of an effect could possibly be related to a limited availability of other essential nutrients as oligo-peptides and/or branched chain volatile fatty acids. In the experiment reported in Chapters 4.1 and 4.2 it was observed that the efficiency of microbial protein in the rumen of sheep fed ammoniated wheat straw supplemented with sugar beet pulp increased after addition of true proteins like casein and potato protein. These protein supplements had a negative effect on rumen degradation of cell wall components, which indicates that fermentative activity of rumen microbes and net microbial protein synthesis are to some extent independent processes. The proportion of microbial protein synthesized in the rumen that actually leaves the rumen, is related to the extent of protozoal predation. The protozoal biomass in the rumen may decrease relatively to the bacterial biomass in response to protein supplementation (Dijkstra, 1993). If protozoal biomass is related to protozoal activity, a reduced protozoal activity for the protein supplemented rations could therefore, at least in part, explain the relatively high efficiency of microbial protein synthesis observed for the rations supplemented with protein.

The increased small-intestinal availability of protein as a result of casein supplementation did not result in an increased DOMI (Chapters 4.1 and 4.2). DOMI from ammoniated wheat straw was completely substituted by that of casein. When protein supplementation to ammoniated wheat straw increased small-intestinal protein availability by 76 % as in the case of potato protein in the experiment with sheep reported in Chapters 4.1 and 4.2, DOMI of the whole ration and that of the ammoniated wheat straw (by 10 %, not significant) increased. In the experiment reported in Chapter 4.3 with cattle, potato protein supplementation did not markedly increase DOMI from ammoniated wheat straw, but DOM from the supplement was consumed additionally.

Regulation of feed intake

The proposed mechanisms of regulation of intake of low-quality feeds range from almost completely physical, stating the rumen processing capacity, *i.e.* holding capacity and turnover, as the major determinant of voluntary intake (e.g. Conrad, 1966; Baile and Forbes, 1974; Bosch et al., 1992) to almost entirely physiological. One possible physiological mechanism of intake regulation of low-quality feeds is based on a balanced availability of ketogenic, glucogenic and aminogenic nutrients for metabolism (e.g. Egan, 1977; Preston and Leng, 1987; see also Chapters 1 and 4.2) with probably the principal basis in the animal behaviour to minimize costs of feed consumption (the noxious effect of oxygen radicals) per unit net energy intake (NEI, Tolkamp and Ketelaars, 1992).

Any theory of feed intake regulation is difficult to invalidate, because of association between various effects of treatments in experiments. Tolkamp and Ketelaars (1992) postulated for example that the NEI-level for minimal oxygen consumption per unit NEI (O_2/NEI) is lower for feeds of a lower quality. Hence, increasing the quality of a feed by ammonia treatment or supplementation could increase the voluntary intake as a result of an increased NEI-level for minimal O_2/NEI. On the other hand, increased quality also means a higher rumen degradation associated with the higher intake in line with a physical intake regulation mechanism. Which of these factors truly determine feed intake awaits the elucidation of causal relationships.

For the various treatments in the experiments reported in this thesis, the effects on voluntary DOMI, parameters associated with rumen processing capacity and parameters associated with metabolic factors potentially affecting feed intake are summarized in Table 2. Rumen processing capacity is determined by the rumen volume, the rate at which feed is comminuted to small particles able to leave the rumen, the fermentative degradation of ingested feed and the rate of passage of undegraded feed particles from the rumen (Chapter 4.4, Bosch et al., 1992). Metabolic regulators of feed intake are represented by the small intestinal protein availability and the efficiency of metabolizable energy utilization (k, Ketelaars and Tolkamp, 1991). Feeds with a higher k-value, as a result of a higher digestibility (ARC, 1980) and/or lower energy expenditure for chewing and metabolization of nutrients, have a higher NEI-level for minimal O_2/NEI (Tolkamp and Ketelaars, 1992). Possible positive effects of an increased protein availability in the small intestine on the efficiency of metabolizable energy utilization (MacRae and Lobley, 1982) have not been taken into account for presentation in Table 2.

The physical intake regulation mechanism is based on the assumption that, in animals fed low-quality feeds, the rumen is filled to the maximum extent, and that increased intake can only occur if the rumen disappearance of particulate matter increases. As Table two shows, passage rates and DOMI were not strongly associated. As discussed in Chapter 4.3 the passage rates were probably not limited by the rates at which particle size reduction occurred, because the majority of particles in the rumen were of such a size that they could potentially leave the rumen. Hence, the increased DOMI due to the experimental treatments

Table 2. Effects of experimental treatments reported in this thesis on DOMI and parameters associated with physical and metabolic intake regulation mechanisms (↑ increase, ↓ decrease, = no change).

Experiment (Chapter)	Treatment	DOMI	Physical regulation				Metabolic regulation	
			Rumen volume	Comminution rate	Rumen degradation	Passage rate	Small intestinal protein availability	k
2.2	Urea supplementation[1]	↑	=	nd	=	↑	↑ (?)	=
3.1 and 3.2	Urea supplementation[1]	=	=	=	=	=	=	=
	Ammonia treatment	↑	↑ (ns)	↑	↑	=	↑	↑
3.3	Ammonia treatment	↑	nd	nd	↑	nd	nd	↑
4.1 and 4.2	Ammonia treatment	↑	↑	nd	↑	=	↑	↑
	Supplementation[2]							
	- casein	=	=	nd	↓	=	↑ (ns)	=
	- potato protein	↑	↑	nd	↓	=	↑	=
4.3	Ammonia treatment	↑	↓	= or ↑	↑	↑	nd	↑
	Supplementation[2]							
	- potato protein	↑	↑	=	=	=	↑ (?)	=

1 supplementation of urea to untreated wheat straw.
2 comparison ammoniated wheat straw plus protein versus ammoniated wheat straw.
nd: not determined
ns: not significant
? not observed, but likely.

could not be explained by increased comminution or passage rates.

Disappearance of particulate matter from the rumen increased for various treatments as a result of an increased fermentative degradation, but, as discussed before, this provides no conclusive evidence for a physical intake regulation mechanism. Under certain experimental conditions, the rumen volume increased as a consequence of the experimental treatment, which implies that the primary assumption of the physical regulation mechanism *i.e.* that the rumen of animals fed low-quality feeds is filled to the maximum extent, was not valid. This was very clearly shown in the experiment reported in Chapter 4.1 where, within individual sheep, a linear relationship between cell wall intake and rumen fluid volume was found. This indicates that these animals tolerated a higher rumen fill under certain conditions. As Table 2 indicates, increased small-intestinal protein availability could well be the factor that allows a higher rumen volume and a higher voluntary intake, in line with the hypothesis suggested by Egan (1977) and Preston and Leng (1987). It was discussed in Chapter 4.2 that supplementation of proteins of a relatively low rumen degradability was required to obtain a sufficiently increased small intestinal availability of a protein and a higher voluntary intake of ammoniated wheat straw. An increased small intestinal protein supply could increase the efficiency of metabolizable energy utilization (MacRae and Lobley, 1982) which, in turn, could be the primary determinant of voluntary feed intake regulation in line with the theory proposed by Tolkamp and Ketelaars (1992).

Species comparison

Figure 1 shows that DOMI per $kg^{0.75}$ per day from straw-based diets was higher for cattle than for sheep. Across species and across breeds within species and within breeds, digestible energy intake (DEI) from low-quality feeds is assumed to be proportional to the maintenance requirements (Graham, 1972; Vercoe and Frisch, 1982; Ketelaars and Tolkamp, 1991). In a direct comparison of wether sheep and steers (Chapter 3.3), DEI from straw based rations was equalized for sheep and cattle if scaled to liveweight (W) to an exponent 0.95 (s.e. 0.015). The exponent calculated for DOMI of diets UWS and AWS plotted in Figure 1 was 0.93 (s.e. 0.024). Though the exponent given in Chapter 3.3 differed significantly from 0.9, the exponent of W to which maintenance requirements of sheep and cattle are related (Graham, 1972), the conclusion would seem justified that energy intake was proportional to maintenance requirements. However, the results of the experiment reported in Chapter 3.3 strongly suggested that maintenance requirements across cattle and sheep were related to W to an exponent lower than 0.9, indicating that cattle were more efficient with regard to deriving maintenance requirements from these straw based rations than did sheep. This is in contrast with observations by Blaxter et al. (1966) and Poppi et al. (1981). However, Bird (1974) concluded from a comparison of various experiments that cattle had relative to their maintenance requirements higher voluntary intakes than sheep. He attributed this to differences in sulphur requirements between the species. Whether this was also the

reason of the species difference as reported in Chapter 3.3 could not be confirmed. In line with the hypothesis that voluntary feed intake from low-quality feeds is determined by net protein availability relative to net energy availability to the tissues, differences in efficiency of microbial protein synthesis or passage of dietary protein in the duodenum could be reasons of the observed difference between species in DEI scaled to maintenance requirements. These parameters could differ between sheep and cattle as a result of the difference in retention time of particulate matter in the rumen. The mean retention time of particulate matter in the rumen was longer in sheep (average of experiments in Chapters 3.1 and 4.1: 31 h) than in cattle (average in experiment in Chapter 4.3: 17 h) in contrast with findings of Poppi et al. (1981) and Hendricksen et al. (1981), who reported longer retention times for cattle than for sheep. A shorter retention time reduces the probability of predation of rumen bacteria by protozoa (Chapter 3.2). A higher efficiency of microbial protein synthesis in cattle compared with sheep could explain why the effect of increased dietary protein flow in the duodenum on straw intake was less in cattle than in sheep (Chapters 4.1 and 4.3).

Statistical analysis of the data plotted in Figure 2 showed no significant differences between sheep and cattle with regard to OMD. In a direct comparison between wether sheep and steers no difference between species with regard to straw digestion could be found (Chapter 3.3). *In sacco* degradation parameters of cell walls, averaged over rations UWS and AWS, differed only slightly between sheep and cattle (sheep: undegradable fraction (U) 24.9 %, k_d 2.3 %/h; cattle: U 22.8 %, k_d 2.8 %/h). As a result of the different retention time of particles in the rumen between species, rumen degradation, described by the formula $k_d*(100-U)/(k_d*k_p)$ (Aitchisson et al., 1986) should be lower in cattle than in sheep. The discrepancy between the observed whole-tract digestion and the prediction of rumen degradation based on k_d and k_p could partly be a result of a higher contribution of hindgut degradation to whole-tract digestion for cattle than for sheep. However, as discussed in by Aitchisson et al. (1986) and in Chapter 4.4, k_d and k_p derived from conventional methods (dacron bag incubations and faecal excretion of Cr-NDF) give biased descriptions of the actual values. The bias between actual values and estimates based on conventional methods could be different for sheep than for cattle. Comparison of k_d- and k_p-values may then not be valid for comparison of intake and rumen processes between species.

Indications of differences in stoichiometry of rumen fermentation between sheep and cattle arose from the results of the experiment reported in Chapter 3.3, where cattle had a higher methane production as a proportion of digestible energy intake (DEI) from straws than sheep. Methane production is associated with rumen production of acetate and butyrate (Czerkawski, 1986). Proportions of acetate and butyrate in the rumen volatile fatty acid pools were, however, 0.81 for sheep (average across UWS and AWS in Chapter 3.1 and 4.1) and 0.83 for cattle (average across UWS and AWS in Chapter 4.3). These values do not support existence of differences in rumen fermentation between sheep and cattle. Methane production in the hindgut is lower than in the rumen (Demeyer, 1991) and a lower contribution of the hindgut to whole-tract digestion for cattle than for sheep could possibly explain the difference in methane production between sheep and cattle. However, the partial digestion in the

hindgut of sheep was low (Chapters 3.1 and 4.1) and it seems not likely that cattle would have a much lower partial digestion in the hindgut.

In conclusion, the effects of the various experimental treatments on voluntary intake and digestion were comparable for sheep and cattle. Digestion of untreated and ammonia-treated wheat straw did not differ significantly between the species, but there were indications that cattle had a higher digestible energy intake relative to maintenance requirements than sheep. The accuracy of k_p and k_d for comparison of experimental treatment effects between sheep and cattle needs to be investigated.

Supplementation and ammonia treatment to increase the nutritive value of wheat straw

A summary of how ammonia treatment and energy and potato protein supplementation improved the nutritive value of the whole rations based on wheat straw in sheep and cattle is presented in Figure 3. In this figure, DOMI of the various rations is expressed relatively to maintenance requirements. Maintenance requirements accepted for sheep were 26 and for cattle 34 g DOMI/$kg^{0.75}$/day (adapted from ARC, 1980). DOMI of the various straw-based rations was compared with that of wilted grass silage fed to sheep as reported by Bosch et al., 1988.

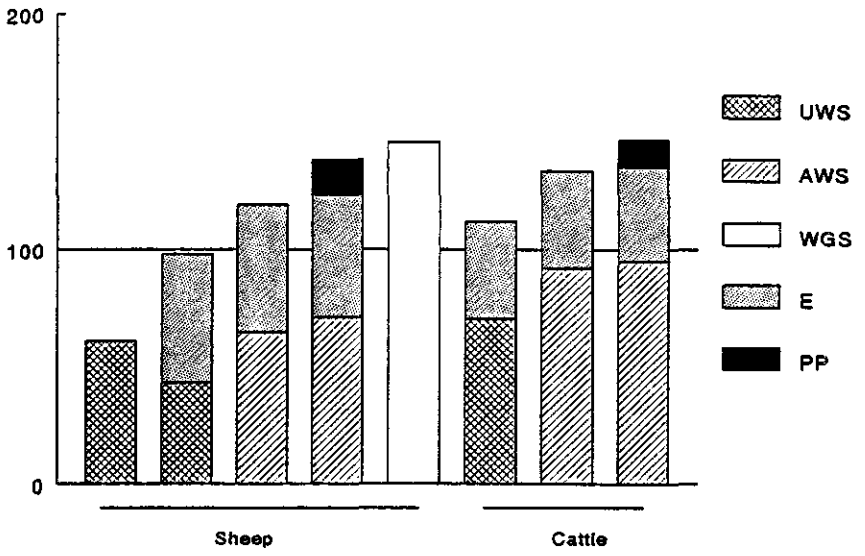

Figure 3. DOMI of rations based on untreated wheat straw (UWS) or ammoniated wheat straw (AWS) compared with that of wilted grass silage (WGS). Maintenance requirements = 100, E = energy, PP = potato protein.

It is shown that rations based on untreated wheat straw could sustain maintenance in sheep as well as in cattle if energy was supplemented. Some production could be achieved from rations based on ammoniated wheat straw, while additional supplementation of potato protein to rations of ammoniated wheat straw and an energy supplement resulted in an energy intake of 1.4-1.5 times maintenance, approximately similar to that of wilted grass silage without energy supplementation.

As indicated in Figure 1, at the levels of energy supplementation as applied in the experiments reported in this thesis, DOMI of straw was highly substituted by that of the energy supplement. Lower energy supplementation levels could therefore result in a similar DOMI from the whole ration, but with a higher proportion of straw, which is likely to be more economically feasible. Increased energy supplementation beyond the levels given in the experiments reported in this thesis will probably result in increased substitution of straw by supplement intake and have only small effects on DOMI and, consequently, on production from these straw-based rations. Whether additional supplementation of proteins of a relatively low rumen degradability to these rations would result in increased DOMI is not known. Doyle et al. (1986) reported no substitution of straw by protein intake when whole cottonseed was supplemented to rice straw for supplementation levels up to 30 % of the total dry matter intake. In many tropical countries, however, protein supplements are relatively expensive, and production levels beyond those possible from a DOMI of 1.4-1.5 times maintenance seem therefore only biologically or economically feasible if wheat straw, even ammonia-treated, as the basal roughage in these rations is replaced by roughage of a higher quality.

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Samenvatting

Gewasresten zijn belangrijk voor de voeding van herkauwers in de Tropen, met name in Zuid-oost Azië. Het is voorts te verwachten, dat produktie van melk, vlees en trekkracht in de tropen in toenemende mate gebaseerd zal zijn op deze gewasresten. Grasland en land, waarop voedergewassen geteeld worden, zal meer en meer benut moeten worden voor de teelt van voedselgewassen voor humane consumptie. Dit wordt deels veroorzaakt door de in snelle mate toenemende bevolkingsdruk, maar "onduurzaam" gebruik van natuurlijke hulpbronnen kan mogelijk leiden tot afnemende bodemvruchtbaarheid, waardoor in de toekomst eveneens meer grond nodig zal zijn om de teruglopende produktiviteit op te vangen. Gewasresten, waarvan stro van rijst, tarwe en mais de belangrijkste representanten zijn in de Tropen, zullen dan ook maximaal benut moeten worden voor de voeding van herkauwers om zo aan de toenemende vraag naar melk, melkproducten en vlees te voldoen. Stro als zodanig is als voedermiddel voor herkauwers van een te lage kwaliteit en zal gesupplementeerd of behandeld moeten worden om groei of melkproduktie te realiseren.

In deze thesis, getiteld: "**Tarwestro als voedermiddel voor herkauwers. Effecten van supplementatie en ammoniak behandeling op vrijwillige voeropname en beschikbaarheid van nutriënten**" worden de resultaten beschreven van een serie experimenten ter identificatie van factoren in tarwestro, die de vrijwillige opname, energievertering en eiwitbeschikbaarheid bij herkauwers beperken, bestudeerd werden. Kennis van deze factoren kan bijdragen tot de formulering van rantsoenen waarin vezelige gewasresten maximaal benut worden.

In hoofdstuk twee worden de effecten van ureum supplementatie op de vertering door pensmicroben en de vrijwillige opname beschreven. Zoals uit een *in vitro* studie (hoofdstuk 2.1) blijkt, kan de beschikbaarheid van stikstof de fermentatieve aktiviteit van pensmicroben beperken. Maximale vertering van de organische stof in een aantal laagwaardige voedermiddelen trad op bij concentraties van ammoniak-stikstof in het substraat van 60-100 mg/l. Deze resultaten bleken echter niet zonder meer te vertalen naar de *in vivo* situatie. Het experiment beschreven in hoofdstuk 2.2, waarin geiten, gevoerd met onbehandeld tarwestro, verschillende nivo's van ureum in de pens geïnfundeerd kregen, gaf aan dat de vertering van het opgenomen stro niet beïnvloed werd door de concentratie van ammoniak-stikstof in de pens. Wel bleek het ammoniak-stikstof-nivo in de pens, wanneer geen ureum geïnfundeerd werd, gepaard te gaan met een lage vrijwillige opname. De resultaten van dit experiment kwamen sterk overeen met die van een aantal soortgelijke experimenten beschreven in de literatuur. In deze experimenten verhoogde ureum infusie de eiwitbeschikbaarheid voor resorptie uit de dunne darm. Dit suggereerde, dat ook in het experiment in hoofdstuk 2.2 de toegenomen vrijwillige opname kon worden toegeschreven aan een toegenomen beschikbaarheid van eiwit voor resorptie uit de dunne darm. Deze toegenomen hoeveelheid darmverteerbaar eiwit zou dan het resultaat moeten zijn van een toegenomen microbiële eiwitsynthese in de pens. In een experiment met schapen (hoofdstuk 3.2) had het infunderen van ureum echter geen effect op de vrijwillige opname noch op de vertering van energie en de efficiëntie van microbiële eiwitsynthese in de pens. De reden voor het mogelijke verschil tussen het experiment met geiten (hoofdstuk 2.2) en dat met schapen (hoofdstuk 3.2) zou

wellicht gelegen kunnen zijn in het feit dat de schapen een energie supplement (bietenpulpbrok) verstrekt kregen, terwijl dit bij de geiten niet het geval was.

In hoofdstuk drie wordt een aantal experimenten beschreven, waarin de effecten van ammoniakbehandeling van tarwestro op vrijwillige opname, vertering en eiwitbeschikbaarheid onderzocht werden. Bij schapen (hamels) en bij koeien (ossen) verhoogde ammoniakbehandeling zowel de vrijwillige opname als de vertering. Het effect van ammoniakbehandeling kon niet worden toegeschreven aan de toegenomen beschikbaarheid van stikstof in de pens (hoofdstukken 3.1 en 3.2). De verschillen in fractionele passage- en verteringssnelheid tussen met ammoniak behandeld en onbehandeld stro waren gering en ook bleek, bij schapen, de bijdrage van het caecum/colon aan de totale celwandvertering voor zowel onbehandeld als met ammoniak behandeld stro betrekkelijk gering te zijn. De toegenomen vertering als gevolg van de ammoniakbehandeling was derhalve vooral veroorzaakt door de toegenomen potentiële verteerbaarheid in de pens. Bij schapen werd gevonden, dat de hogere opname van met ammoniak behandeld stro geassocieerd was met een groter (hoewel niet significant) pensvolume en een kortere herkauwtijd per kg opgenomen celwanden. Dit laatste resulteerde in een groter aandeel van grotere partikels in de pens. De efficiëntie van de microbiële eiwitsynthese in de pens van schapen was laag voor zowel behandeld als onbehandeld stro, waardoor de beschikbaarheid van eiwit voor resorptie uit de dunne darm ook laag was. Een vergelijking van de opname en benutting van energie uit behandeld en onbehandeld tarwestro door hamels en ossen (hoofdstuk 3.3) liet zien, dat er geen verschillen tussen de diersoorten bestonden wat de vertering en metabolizeerbaarheid van bruto energie en efficiëntie van benutting van metabolizeerbare energie betreft, maar er waren sterke aanwijzingen dat de ossen relatief ten opzichte van hun onderhoudsbehoefte meer verteerbare energie opnamen dan de hamels. De metabolizeerbaarheid als proportie van de verteerbare energie was voor beide diersoorten en voor alle rantsoenen in dit experiment ongeveer 80 % en het leek aannemelijk, dat de efficiëntie van benutting van metabolizeerbare energie uit alle *ad libitum* opgenomen rantsoenen ongeveer 60 % was.

In hoofdstuk vier worden twee experimenten beschreven waarin eiwitsupplementen toegevoegd werden aan met ammoniak behandeld stro. In de hoofdstukken 4.1 en 4.2 worden de resultaten weergegeven van een experiment met schapen, waarin caseïne, een eiwit, dat in hoge mate door microben in de pens afgebroken wordt, en aardappeleiwit, dat voor ongeveer 50 % in de pens afbreekbaar is, gesupplementeerd werden. Supplementatie met aardappeleiwit resulteerde in een toegenomen totale energieopname, terwijl de opname van stro gesubstitueerd werd door die van caseïne. Beide eiwitten hadden een negatief effect op de vertering van celwandbestanddelen in de pens, caseïne meer dan aardappeleiwit. Deze lagere pensvertering werd voor een deel gecompenseerd door een hogere vertering in het caecum/colon. Het vloeistofvolume in de pens nam toe met het nivo van celwandopname, terwijl er geen effect van de experimentele behandelingen op de fractionele passage snelheid uit de pens gevonden werd. Dit suggereerde, dat andere factoren dan het volume en de verwerkingscapaciteit van de pens de vrijwillige voeropname bepaalden.

In deze experimenten bleek een lineair verband te bestaan tussen de werkelijk uit de dunne darm geresorbeerde hoeveelheid eiwit en de opname van verteerbare organische stof. Omdat de efficiëntie van benutting van het geresorbeerde eiwit constant was over de

rantsoenen (0.54) en ook de efficiëntie van benutting van verteerbare organische stof voor netto energie constant verondersteld mag worden voor *ad libitum* gevoerde rantsoenen, zou een lineaire relatie tussen eiwitbeschikbaarheid op het nivo van de dunne darm en de opname van verteerbare organische stof terug te voeren zijn op een evenwicht tussen netto eiwit- en netto energie-beschikbaarheid voor metabolische processen.

Ook bij ossen werd behandeld stro gesupplementeerd met aardappeleiwit (hoofdstuk 4.3). Ook hier werd gevonden, dat de totale opname aan verteerbare organische stof toenam (hoewel niet significant). Opname van celwanden, pensvolumina en snelheid van deeltjesverkleining, microbiële degradatie en passage bleken echter niet beïnvloed te zijn door de aardappeleiwitsupplementatie. Een pensmodel, gebruikt om de snelheidsconstanten bepaald in het experiment weergegeven in hoofdstuk 4.3 te valideren, gaf aan, dat de gebruikte methodes ter bepaling van de fractionele passagesnelheid van kleine deeltjes uit de pens (uitscheiding van een merkstof in de mest) en de fractionele degradatiesnelheid in de pens (incubatie van voer in dacron zakjes) geen goede beschrijving van de actuele snelheidsconstanten gaf. Analyse van de gevoeligheid van de modelvoorspellingen van de verteerbare celwandopname voor veranderingen van de parameters in het model gaf aan, dat de potentiële verteerbaarheid, het pensvolume en de verteringssnelheid volgens het model de meest limiterende factoren waren voor verteerbare celwandopname. Verhoging van de passagesnelheid of de snelheid waarmee deeltjes verkleind worden door herkauwen had slechts een marginaal effect op de voorspelling van de verteerbare celwandopname.

In hoofdstuk 5 tenslotte, worden de resultaten van de verschillende experimenten geïntegreerd. Door de verschillende nivo's van energiesupplementatie, die in de experimenten werden toegepast was het mogelijk een (ruwe) indicatie te krijgen van het effect van energiesupplementatie op de opname van verteerbare organische stof (DOMI). Bij relatief hoge nivo's van energiesupplementatie (10-20 g DOMI/$kg^{0.75}$/dag) bleek de DOMI van stro compleet gesubstitueerd te worden door die van het energiesupplement. Dit impliceert, dat hogere DOMI van op stro gebaseerde rantsoenen alleen te bereiken is door supplementatie van een eiwit met een lagere afbreekbaarheid in de pens, waarbij geen substitutie van stro-door supplement-opname gevonden werd. Tot welke nivo's van supplementatie van eiwitten met een lage afbreekbaarheid geen substitutie van de stro-opname optreedt zou in vervolgonderzoek duidelijk gemaakt moeten worden. Bij het nivo van aardappeleiwitsupplementatie, zoals toegepast in de experimenten beschreven in deze thesis, kon van de rantsoenen bestaande uit behandeld stro en een energie- en eiwitsupplement 1.4-1.5 maal de onderhoudsbehoefte opgenomen worden.

Curriculum vitae

Simon Jenne Oosting werd op 7 oktober 1959 geboren te Scharsterbrug (Friesland). In 1978 behaalde hij het Gymnasium β-diploma aan het Christelijk Gymnasium te Leeuwarden. In datzelfde jaar begon hij zijn studie Zoötechniek aan de toenmalige Landbouwhogeschool in Wageningen. Hij haalde zijn doctoraal in juli 1985 met als hoofdvak Tropische Veehouderij en als bijvakken Veevoeding, Graslandkunde en Ontwikkelingseconomie. Zijn praktijktijd bracht hij in 1982 door in Colombia. In 1985 trad hij in dienst van de vakgroep Tropische Veehouderij van de Landbouwuniversiteit als medewerker van het "Indo-Dutch project on bioconversion of crop-residues" in India. Vanaf 1987 raakte hij in toenemende mate betrokken bij in Wageningen uitgevoerd onderzoek naar de waarde van gewasresten als voeder voor herkauwers, hetgeen in 1989 resulteerde in een aanstelling bij de sectie Tropische Veehouderij van de vakgroep Veehouderij voor het uitvoeren van de Wageningse inbreng in het door de EEG gefinancierde project: "Utilization of crop-residues and supplementary feeds in tropical developing countries".