

**AN EVALUATION OF DEGRADABLE PROTEIN AND
NONPROTEIN NITROGEN ON INTAKE AND DIGESTION
BY DOHNE MERINO SHEEP FED WHEAT STRAW**

by

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Pectora laborent cultus recti

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

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ABSTRACT

An Evaluation of Degradable Protein and Nonprotein Nitrogen on Intake and Digestion by Dohne Merino Sheep fed Wheat Straw

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South Africa, like many other developing countries throughout the world, has a rapidly growing human population, resulting in a consequent increased demand for food. Ironically, this increased food supply has to be produced on a smaller area of available agricultural land, which means that agricultural production processes have to become more efficient. Furthermore, the majority of the people in these countries are unschooled and poverty is a common phenomenon. Therefore, animal scientists are faced with the challenge and the responsibility to provide affordable, high quality food to these people.

One way of reaching this objective is to improve the utilisation of low-quality, high roughage feedstuffs like crop residues and dry natural grass pastures. In the winter rainfall area of South Africa alone, about 460 000 ha of wheat straw are annually available. The ruminant animal has the ability to utilise the relatively unavailable energy (cellulose, hemi-cellulose and pectin) in the fibre component of these low-quality forages. Unfortunately, various factors, of which a N deficiency is the pre-dominant one, limit the utilisation of these feedstuffs. If the ability of the ruminant to utilise low-quality, fibrous energy sources is improved, these abundantly available and relatively inexpensive crop residues and natural pastures can be converted into high quality protein food for human consumption.

Therefore, in order to rectify the N deficiency caused by these low-quality forages, the supplemental N requirement to optimise the fermentation and digestive processes of the ruminant animal has to be determined. The first study was conducted to determine the supplemental rumen degradable protein (RDP) requirement, to maximise the digestible organic matter intake (DOMI) of Dohne Merino sheep fed wheat straw. Keeping the high cost of natural protein supplementation in mind, the purpose of the second study was to determine the amount of true protein that can be replaced by nonprotein nitrogen (NPN) in RDP supplements fed to Dohne Merino sheep consuming wheat straw.

In both trials animals had *ad libitum* access to low-quality wheat straw (3.2% CP; 74.2% NDF) and water. In the first trial, RDP (calcium caseinate: 90% CP; 100% rumen degradable) was intraruminally administered at 07h00 and 19h00, at the following levels: 0, 40, 80, 120 and 160 g/d. Intake, fermentation and digestion were monitored to determine the RDP requirement to maximise DOMI. Digestible organic matter (OM) intake displayed a quadratic increase with elevated amounts of RDP ($P < .01$), and was maximised at an estimated 3.15 g RDP/kg BW^{.75} or 11.6% of DOM. Forage OM intake tended to increase quadratically ($P = .15$) with higher RDP levels. Microbial nitrogen (MN) flow to the duodenum and microbial efficiency increased quadratically ($P \leq .04$) and fluid dilution rate tended to increase in a quadratic manner ($P = .15$) with increased RDP supplementation levels. Ruminal ammonia nitrogen (NH₃-N) and total volatile fatty acid (VFA) concentrations increased linearly ($P \leq .07$), while rumen pH exhibited a variable response to increased RDP levels (cubic; $P = .08$). Increasing RDP supplementation to Dohne Merino wethers consuming wheat straw, generally enhanced forage utilisation and DOMI.

In the second trial, urea replaced different levels of casein N on an isonitrogenous basis, ranging from 0 - 100%. Since true protein is much more expensive than urea, the purpose of this study was to determine the maximum natural protein level that can be replaced by urea in RDP supplements, without adversely affecting intake and/or fermentation and digestive processes. The control treatment provided all of the RDP in the form of calcium caseinate (90% CP; 100% rumen degradable). The percentages of supplemental RDP from urea in the other treatments were 25, 50, 75 and 100%. The 100% urea treatment was balanced with maize starch to contain 40% CP and all other treatments received the same amount (150 g/d) of starch. Intake of forage OM showed a weak decreasing trend (linear; $P = .16$) with

increasing urea levels. Ruminant digestibilities of OM and NDF were not affected ($P \geq .18$) by urea level. Increasing urea levels resulted in linearly reduced total tract OM and NDF digestibilities ($P \leq .10$). As a result, DOMI declined (linear; $P < .01$) with increasing proportions of urea. Effects of increasing urea proportions on duodenal N flow, microbial efficiency and fluid dilution rate were minimal. Ruminant $\text{NH}_3\text{-N}$ tended to increase quadratically with increasing urea levels ($P = .14$). Total VFA concentration decreased linearly ($P = .03$), while rumen pH increased in a linear manner ($P = .08$) with increasing urea proportions. Branched-chain volatile fatty acids (BCVFA's) and valerate decreased linearly ($P \leq .05$) with increasing urea levels, while other VFA's and the acetate:propionate ratio were generally not affected by treatment ($P \geq .16$). It appears as though ruminant and total tract OM and NDF digestibility criteria, as well as DOMI reached maximum values at substituting 25% of casein for urea. It is therefore concluded that replacing 25% of casein with urea in RDP supplements, will maintain effective utilisation of low-quality forages by sheep.

SAMEVATTING

`n Evaluasie van Degradeerbare Proteïen en Nie-proteïen Stikstof op Inname en Vertering van Koringstrooi deur Dohne Merino Skape

deur

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Ontwikkelende lande, insluitend Suid-Afrika, word gekenmerk deur `n vinnige bevolkingsaanwas. Dit lei tot `n verhoogde vraag na voedsel, wat op `n gevolglik kleinerwordende beskikbare kommersiële lanbbou-area geproduseer moet word. Die doeltreffendheid van produksieprosesse in die landbousektor moet dus noodgedwonge verbeter word. Die oorgrootte meerderheid van die bevolkings in ontwikkelende lande is ongeletterd en armoede en hongersnood is soms `n algemene verskynsel. Veekundiges word dus gekonfronteer met die uitdaging en verantwoordelikheid om bekostigbare, hoë kwaliteit voedsel aan hierdie mense te voorsien.

Een van die mees doeltreffende metodes om hierdie probleem aan te spreek, is om die benutting van lae kwaliteit, hoë-vesel voerbronne, bv. oesreste en droë grasweidings te verhoog. In die winterreënstreek van Suid-Afrika alleen, is 460 000 ha koringstrooi jaarliks beskikbaar. Herkouers beskik oor die besondere vermoë om die relatief onbeskikbare energie (sellulose, hemi-sellulose en pektien) in die veselkomponent van hierdie lae kwaliteit voere te benut. Verskeie faktore, waarvan `n N-tekort die mees prominente is, beperk egter die benutting van hierdie voerbronne. Die uitdaging is dus om die herkouer se vermoë om hierdie voere te benut, te optimaliseer. Sodoende word `n geredelik beskikbare, onderbenutte en relatief goedkoop voerbron omgeskakel in hoë kwaliteit proteïen vir menslike gebruik.

Die N-aanvullingsbehoefte om die fermentasie- en verteringsprosesse van die herkouerdiere te optimaliseer moet dus bepaal word, sodat die N-tekort in herkouers, wat soortgelyke weidings benut, reggestel kan word. Gevolglik was die doel met die eerste proef om die behoefte aan rumen degradeerbare proteïene-(RDP)-aanvulling, vir die maksimum inname van verteerbare organiese materiaal (VOM) van Dohne Merino skape wat koringstrooi ontvang, te bepaal. Weens die hoë koste van natuurlike proteïenaanvulling, was die doel met die tweede proef om die hoeveelheid ware proteïene in RDP-aanvullings, vir Dohne Merino skape wat koringstrooi ontvang, wat met nie-proteïene stikstof (NPN) vervang kan word, te bepaal.

In beide eksperimente het die diere *ad libitum* toegang tot koringstrooi (3.2% RP; 74.2% NBV) en water gehad. In die eerste proef is RDP (kalsiumkaseïnaat; 90% RP; 100% rumen degradeerbaar) teen 07h00 en 19h00 intraruminaal toegedien, teen die volgende peile: 0, 40, 80, 120 en 160 g/d. Inname, fermentasie en vertering is gemonitor om die RDP behoefte vir die maksimum inname van VOM te bepaal. Veteerbare OM-inname het 'n stygende kwadratiese tendens ($P < .01$) getoon met verhoogde RDP-peile en het 'n maksimum bereik by 'n aanvullingspeil van 3.15 g RDP/kg metaboliese liggaamsmassa ($LM^{.75}$) of 11.6% van VOM. Organiese materiaalinnames vanaf koringstrooi het geneig om kwadratiese toe te neem ($P = .15$) met verhoogde RDP-peile. Mikrobiële stikstof-(MN)-vloei na die duodenum en mikrobiële effektiwiteit het kwadratiese toegeneem ($P \leq .04$) en vloeistof deurvloeitempo het 'n neiging vir 'n kwadratiese toename ($P = .15$) met verhoogde RDP-peile getoon. Rumen-ammoniakstikstof (NH_3-N) en vlugtige vetsuur-(VVS)-konsentrasies het lineêr toegeneem ($P \leq .07$), terwyl rumen pH 'n wisselende reaksie (kubies; $P = .08$) met stygende RDP-peile getoon het. Veteerbare OM-inname en benutting van koringstrooi is verbeter deur stygende peile van RDP aanvulling by Dohne Merino hamels.

In die tweede proef is kaseïene op 'n iso-stikstof basis met verskillende ureumpeile, vanaf 0 - 100%, vervang. Omdat ureum baie goedkoper is as natuurlike proteïene, was die doel van die tweede proef om die hoeveelheid natuurlike proteïene in RDP-aanvullings te bepaal wat met ureum vervang kan word, sonder om inname, fermentasie en vertering te benadeel. Die kontrolebehandeling het 100% van die RDP in die vorm van kalsiumkaseïnaat (90% RP; 100% rumen degradeerbaar) voorsien, terwyl ureum in die ander behandelings onderskeidelik 25, 50, 75 en 100% van die kaseïene-N vervang het. Die 100% ureumbehandeling is met meliëstysel tot 40% RP gebalanseer en dieselfde hoeveelheid stysel (150 g/d) is by al die

ander behandelings ingesluit, om moontlike effekte van stysel op rumenfermentasie te elimineer. Organiese materiaalinnam vanaf koringstrooi het 'n swak dalende tendens getoon met stygende ureum insluitingsvlakke (lineêr; $P = .16$). Rumenverteerbaarheid van OM en neutraal bestande vesel (NBV) is nie deur die ureumpeil beïnvloed nie ($P \geq .18$). Toenemende ureumpeile het gelei tot 'n lineêre afname in totale kanaal OM en NBV-verteerbaarheid ($P \leq .10$). Gevolglik het VOM innam lineêr afgeneem ($P < .01$) met stygende ureum insluitingspeile. Die effek van behandeling op duodenale N-vloei, mikrobiese effektiwiteit en vloeistof deurvloeiempo was minimaal. Rumenammoniakstikstof-($\text{NH}_3\text{-N}$)-konsentrasie het 'n stygende kwadratiese tendens getoon ($P = .14$) met toenemende ureum peile. Totale vlugtige vetsuur-(VVS)-konsentrasies het lineêr gedaal ($P = .03$), terwyl rumen pH lineêr toegeneem het ($P = .08$) met stygende ureum insluitingsvlakke. Vertakte ketting VVS'e en valeriaansuurkonsentrasies het lineêr gedaal ($P \leq .05$) met stygende ureumpeile, terwyl die ander VVS'e en die asynsuur:propioonsuurverhouding oor die algemeen nie deur behandeling beïnvloed is nie ($P \geq .16$). Dit blyk asof VOM innam, sowel as rumen- en totale kanaal verteerbaarheidsmaatstawwe, by 25% vervanging van kaseïen-N met ureum-N 'n maksimum bereik het. Die gevolgtrekking is gemaak dat die doeltreffende benutting van lae kwaliteit ruvoere deur skape, gehandhaaf sal word deur 25% van die ware proteïen in RDP-aanvullings met ureum te vervang.

*Aan my ouers, Willie en Corlia Nolte. Julle liefde en ondersteuning
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LIST OF ABBREVIATIONS

ADF	- acid detergent fibre
AIA	- acid insoluble ash
BCVFA`s	- branched-chain volatile fatty acids
BW	- body weight
BW ^{.75}	- metabolic body weight
C	- carbon
CP	- crude protein
Cr	- chromium
DE	- digestible energy
DM	- dry matter
DMI	- dry matter intake
DOMI	- digestible organic matter intake
EDTA	- ethylenediaminetetra-acetic acid disodium salt
g/d	- gram per day
h	- hour
HCl	- hydrochloric acid
HCHO	- formaldehyde
ME	- metabolisable energy
MJ	- megajoule
MN	- microbial nitrogen
MP	- microbial protein
N	- nitrogen
NaCl	- sodiumchloride
NAN	- nonammonia nitrogen
NaOH	- sodium hydroxide
NDF	- neutral detergent fibre
NH ₃	- ammonia
NH ₃ -N	- ammonia nitrogen
NMNAN	- nonmicrobial-nonammonia nitrogen
NPN	- nonprotein nitrogen
OM	- organic matter

P	- phosphorus
P/E	- protein/energy ratio
RDP	- rumen degradable protein
RUP	- rumen undegradable protein
TDN	- total digestible nutrients
VFA's	- volatile fatty acids

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CHAPTER 1

General Introduction

Because production of food crops has priority over that of animal feedstuffs, low-quality roughages (< 7% CP), especially crop residues like cereal straws, are the predominant energy sources available to ruminants for considerable parts of the year. Europe annually produces about 330 million tons of these residues (Cañeque *et al.*, 1998), and in the winter rainfall region of South Africa about 460 000 ha of wheat stubble are available annually (Brand, 1996). The primary nutrient sources in these residues are the structural carbohydrates in the cell walls, i.e. cellulose, hemicellulose and pectin. Another cell wall component, lignin, is not digestible by rumen microbes and therefore has no potential energy value. In contrast to humans and monogastric animals, ruminants have the ability to ferment these carbohydrates in the rumen through microbial action and consequently utilise the resultant released sources of energy. This energy is in the form of volatile fatty acids (VFA's), of which acetate, propionate and butyrate are the pre-dominant ones.

These low-quality roughages are usually unpalatable, fibrous and often deficient in nitrogen (N), phosphorus (P), vitamin A and, possibly, trace minerals (NAS, 1971), which often cause them to be insufficient in providing enough digestible energy (DE) to meet the maintenance requirements of grazing ruminants (Dias-da-Silva, 1986). Furthermore, Fujihara & Nakao (1982) suggested that animals may have more difficulty in forming a bolus as the fibre content of the forage increases, which affects both eating and rumination behaviour. However, these residues are readily available, is a relatively inexpensive feed source and holds considerable amounts of potential digestible energy. Considering the growing world population and consequent increase in demand for food, we face the challenge of producing more food on the same, or even smaller area of agricultural land. This means that production processes have to become more efficient, and therefore a re-examination of these poor quality roughages for maximum exploitation of its energy for practical livestock production, is inevitable.

Efficiency in utilisation of low-quality roughages can be increased by providing protein and/or energy supplements to grazing animals, and thus maintain optimum rumen ammonia concentrations for maximum microbial growth and fibre digestion. Provision of energy supplements has in general been ineffective in improving the energy status of cattle and sheep grazing low-quality forage (Ammerman *et al.*, 1972; DelCurto *et al.*, 1990; Sanson *et al.*, 1990). The reason for this is probably that the animal substitutes the less digestible and unpalatable roughages for the more readily digestible energy supplements. The provision of energy supplements to ruminants grazing low-quality roughages also does not address the essence of the problem, because these forages already contain considerable amounts of metabolisable energy, primarily as cellulose. Although cellulose is not easily fermented, the cellulolytic rumen bacteria can metabolise the relatively unavailable cellulolytic energy if there is not a N deficiency in the rumen. It is thus clear that energy is not the first limiting nutrient in low-quality roughages. In support of this view Elliott & Topps (1963) concluded that when cattle are fed protein sufficient for maintenance, they will eat enough roughage of low-quality (3.4% CP in their study) to satisfy their energy requirements for maintenance. One can therefore assume that sheep will exhibit the same characteristic and increase their voluntary intake of low-quality roughages to a level where their energy requirements for maintenance will be met, despite the unpalatability and low digestibility of these kinds of forages, provided that sufficient protein is supplemented. Therefore, the answer towards a more efficient and economically beneficial utilisation of low-quality roughages seems not to be supplementation with energy, but to improve the fermentation and digestion processes of the animal via protein supplementation, in order to utilise the natural energy within the forages.

With this in mind and because N is generally viewed as the first limiting nutrient for ruminants grazing low-quality forage (Kempton & Leng, 1979), protein supplementation seems to be the solution towards maximum exploitation of the relatively indigestible energy fraction in low-quality roughages. In contrast to energy supplements, protein supplementation has increased dry matter intake (DMI) as a result of an increased rate of digestion and/or passage (Ellis, 1978; Church & Santos, 1981; DelCurto *et al.*, 1990) and possibly via improvement in the efficiency of metabolisable energy (ME) utilisation. According to Kartchner (1980), responses to supplemental protein are usually observed when the CP content of the forage is less than 6 to 8%. It is thus clear that the fermentation and

digestion processes of grazing ruminants will be enhanced through effective protein supplementation in order to improve livestock performance.

It is essential to distinguish between the different CP fractions, i.e. rumen degradable protein (RDP) and rumen undegradable protein (RUP). Rumen undegradable protein is particularly effective in improving livestock performance (especially during growth, late pregnancy and lactation) because it is not fermented in the rumen, but is catabolised in the lower alimentary tract to form amino acids which are then absorbed and incorporated into muscle, milk or wool. In this way many essential amino acids reach the lower digestive tract instead of being fermented in the rumen and incorporated into microbial protein (MP), which does not always provide the ideal amino acid profile in the lower digestive tract. However, RUP will not serve the purpose of effectively enhancing the ruminal fermentation process on low-quality, N deficient forages, because it does not provide N to the rumen microbes. Because of the N deficiency of low-quality roughages, the rumen microbial populations of ruminants grazing these forages have a very low activity and consequently cannot utilise the relatively indigestible energy sources. Rumen degradable protein, on the other hand, is fermented in the rumen and is broken down to amino acids, peptides and ammonia (NH_3), which serve as nutrients for the rumen microbes. Peptides and amino acids can be directly incorporated into MP (Nolan *et al.*, 1976), which increases the efficiency of MP production, as well as production rate. Providing these nutrients to the microbes will enhance their activity and enable them to utilise the energy fraction of the roughages more efficiently, which in turn will lead to a higher microbial population growth and increased fermentation rate in the rumen. Hannah *et al.* (1991) concluded that forage intake and digestion of low-quality forage by cattle will be enhanced by providing adequate amounts of supplemental protein.

Another source of N to the rumen microbes is non-protein nitrogen (NPN), primarily urea, which only provides NH_3 to the rumen microbes. Although 80% of rumen bacteria and protozoa can grow with NH_3 as their sole source of nitrogen, peptides and amino acids can be directly incorporated into MP, with a resultant benefit in rumen microbial growth efficiency (Baldwin & Allison, 1983). A higher microbial growth efficiency will inevitably lead to an increased digestion rate. This in turn will lead to an increased digesta passage rate and a subsequent increase in DMI, which will enhance animal performance.

Urea is very unpalatable and will definitely have a lower stimulatory effect on voluntary intake than RDP, but in practice it is possible to disguise the unpalatability with other components of the supplementary diet, if the urea levels are not too high. Furthermore, urea is fermented very quickly, which may lead to excessive amounts of NH_3 being released in the rumen immediately after consumption of the supplement. This would decrease the efficiency of urea supplements, because a part of the available NH_3 cannot be utilised quickly enough by the rumen microbes and is absorbed through the rumen wall. This NH_3 is transported to the liver where it is converted to urea and recycled to the rumen, mainly by means of saliva (Doyle *et al.*, 1982), where it serves as a source of NH_3 and is utilised by the microbes. However, because of the inverse relationship between the level of protein intake and blood urea-N entry into the rumen (Bunting *et al.*, 1987), a great deal of this absorbed NH_3 will be excreted in the urine as urea. Energy is used for the excretion of additional NH_3 which increases the maintenance requirement of the animal.

According to Egan (1974) there is a net gain of non-ammonia N (NAN) anterior to the small intestine when sheep are fed poor quality dried roughages. The assumption can be made that the additional NAN is mainly derived from the conversion of recycled urea N into MP. However, MacRae *et al.* (1979) found that only 0.9 to 1.1g N from a total gain of 3.3 to 3.7g NAN day⁻¹ was derived from urea-N recycled to the rumen, the remainder was accounted for as non-urea endogenous NAN.

Furthermore, excessive amounts of urea during a short period can lead to urea toxicity which can result in the death of the animal (Helmer & Bartley, 1971; Fonnebeck *et al.*, 1975). Due to the unpalatability of urea it is not likely that animals will eat too much of it, but the possibility exists, because palatable products are often included in the supplements to disguise the taste of urea and increase intake of the supplement.

In contrast, RDP is fermented more slowly in the rumen and provides nutrients to the rumen microbes on a more regular and continuous basis than NPN. For these reasons RDP is more efficient in improving the utilisation of low-quality forages than NPN. However, NPN is less expensive than RDP per unit N and according to Campling *et al.* (1962) it increases digestibility and voluntary intake of oat straw by cows, due to an improved carbohydrate

digestibility. Therefore, it is important to determine to what extent urea can substitute RDP in protein supplements on low-quality forages without sacrificing animal performance.

As already mentioned, both protein and urea supplementation to ruminants grazing low-quality roughages will increase voluntary intake and organic matter (OM) digestion (Campling *et al.*, 1962; Egan, 1965; Church & Santos, 1981; Egan & Doyle, 1985; Dias-da-Silva & Sundstøl, 1986; Krysl *et al.*, 1987; Hannah *et al.*, 1991; Mawuenyegah *et al.*, 1997) and thus animal performance, but presumably not to the same extent or by means of improving the same components of the digestive process. Therefore, in order to improve animal performance, it is necessary to thoroughly understand the regulatory processes that control intake.

Through the years there have been contrasting views concerning the regulation of voluntary feed intake by ruminants. Blaxter *et al.* (1961), stated that the amount of food consumed by sheep is determined by the capacity of their digestive tracts and that physical factors rather than physiological factors regulate appetite. The reason for this appears to be that sheep eat to a constant distension of their digestive tracts, measured by the “fill”, which is in turn determined by the passage rate of the feed and its digestibility. The specific distension of the digestive tract at which the sheep will stop eating seems not to be all that constant, since cottonseed meal supplementation increased prairie hay intake in mature ewes by expanding gastrointestinal fill rather than increasing particulate passage rate (Krysl *et al.*, 1987). In addition, Blaxter *et al.* (1961) reported that voluntary intake of long fodders is related to the apparent digestibility of their energy, increasing rapidly as digestibility increased from 38% to 70% and thereafter more slowly.

Egan (1972) found that the protein/energy (P/E) ratio (g digestible protein/MJ digestible energy) was much more dominant in regulating voluntary intake of roughages, than digestibility of OM. Where P/E ratios in digestion products are less than 5.5g digestible protein/MJ DE, responses in voluntary intake of roughage diets due to supplemental protein digested in the intestine may be expected. The reason for this is that increases in voluntary intake are usually the result of rectifying a deficiency in the availability of nitrogen to the micro-organisms in the reticulo-rumen, with a consequent increase in the rate of removal of digesta by fermentation and outflow (Egan & Doyle, 1985). If the P/E value is greater than

7.5, the limitation to intake lies in factors other than protein inadequacy, probably physical factors such as space-occupying effects of the digesta load associated with a low fibre digestion rate. In this regard Crampton *et al.*, (1957) have found that voluntary intake of fodders is a better index of their nutritive value than either their chemical composition or total digestible nutrient (TDN) content.

Plant cell wall constituents (CWC) often comprise the largest proportion of the OM in the diet and their concentration in OM is generally inversely related to intake. Therefore, the extent of digestion of CWC and their resistance to particle size reduction in the reticulo-rumen appear to be key factors in the regulation of feed intake (Egan & Doyle, 1985). However, in the same study it was found that the infusion of urea into the rumen of sheep offered a roughage diet, significantly increased the voluntary intake of the diet with no clear evidence of a change in the digestibility of OM or in the digestion rate of CWC. It can thus be concluded that the increase in intake was mediated through the effects of additional intestinally digested MP on the N status of the host animal, rather than through the effect of N on the fermentation activities of the microbes themselves. For this reason N status of the animal is a factor contributing to the regulation of voluntary consumption of low-protein roughages by sheep (Egan, 1965; Egan & Moir, 1965).

Physical factors like the capacity of the digestive tract, the digestibility of the diet and the passage rate of digesta through the alimentary tract, rather than physiological factors have been suggested as feed intake regulators (Egan, 1965). However, Conrad *et al.* (1964) suggested that physical and physiological factors change relatively in importance as the digestibility of dietary materials increases. At higher levels of digestibility (67-80%) physiological factors such as metabolic body size and level of production seemed to be the primary regulators of feed intake, whereas in medium to good quality forages (55-67% digestibility) physical factors appeared to play a dominant role in regulating intake. With low-quality fodders, intake again appeared to be related to physiological factors. It is clear that the regulation of voluntary intake is a complex process and that both physical and physiological regulatory factors must be considered when the objective is to increase feed consumption.

Another factor that has an influence on voluntary feed intake is forage quality and more specifically the fibre fractions. Material with a NDF content above 55-60% has been found to depress intake (Meissner *et al.*, 1991). It is a well known fact that higher quality forages stimulate voluntary intake in comparison to low-quality forages which inhibit intake and consequently restrain animal performance. A possible explanation for this is that high-quality forages contain higher levels of metabolisable protein and energy and less indigestible fibre components. Therefore, it is to be expected that the response to protein supplementation of animals being fed low-quality forages will be greater than those of animals being fed high-quality, more palatable diets. In this regard Lee *et al.* (1987) reported a greater increase in forage consumption in response to N supplementation for lower quality hays when forages of similar origin, but with different N contents were compared. Köster (1995) also confirmed that the response to protein supplementation was much greater when the CP content of the forage was less than 3% than when it was between 3 and 6%.

A further contributing factor towards feed intake regulation is the particle size of the forage. Alwash & Thomas (1974) found depressions in ruminal digestion of OM and fibre due to decreased rumen retention times associated with greater feed intake or smaller forage particle size. Usually a faster passage rate will lead to an increased voluntary intake. However, Firkins *et al.* (1986) found no differences in OM intake and duodenal OM flow between ground- and chopped hay diets, but apparent ruminal OM digestion and percentage of digestible OM disappearing in the rumen were greater for ground- than for chopped-hay diets. It was concluded that the greater surface area per gram DM of ground hay should allow more rapid colonisation by rumen microbes and, subsequently more extensive fermentation of the ground vs the chopped hay diet.

Campling *et al.* (1962) found, in cows, that voluntary intakes of hay, oat straw and oat straw with urea, were inversely related to the mean retention times of feed residues in the reticulo-rumen. Therefore it seems likely that factors affecting the rate at which feed particles are reduced to a size suitable for transfer to the omasum will largely determine their mean retention time in the reticulo-rumen, the mean OM flow rate from the reticulo-rumen and hence the voluntary intake of roughage diets (Freer *et al.*, 1962).

The two competitive processes of reduction in particle size and passage of small particles determine fermentation time and are modulated by the animal through ingestive chewing, ruminative chewing and passage from the rumen (Wilson & Kennedy, 1996). Under marginal conditions when availability of food is limited, a ruminant reduces the force or frequency of its ruminal contractions, which prolongs the retention time of feed particles in the rumen and thereby maximises the digestive recovery of nutrients per weight of food. In contrast, ruminants fed on adequate amounts of low-quality fibrous diets, as was the case in our study, maximise nutrient yield by increasing rumination and rate of passage (Kennedy & Doyle, 1993, as cited by Mawuenyegah, 1997). In support of this view, Merchen *et al.* (1986) have shown that wethers fed at a high intake level, apparently digested a greater quantity (g/d) of OM than when fed at a lower intake. These authors also found that the proportion of total OM digested (% of digestible OM) decreased with increasing intake levels, presumably as a result of an increased passage rate, since the OM flow at the duodenum was increased at higher intake levels.

It is important to realise that an increased feed intake will result in a faster passage rate, which in turn will lead to a decreased rumen retention time and a consequent depression in ruminal digestion of OM and fibre (Firkins *et al.*, 1986). For this reason, maximising intake will not necessarily maximise animal performance. However, an increased intake will stimulate microbial population growth in the rumen because of a higher substrate availability and consequently improve rumen fermentation, which in turn will lead to more MP being synthesised. The additional MP plus increased amount of dietary protein that escapes rumen fermentation due to a faster passage rate, supply more digestible protein in the small intestine and should improve the N-status of the animal, which would enhance voluntary feed intake. An increased feed intake will result in an increased production of VFA's and absorption of nutrients from the digestive tract (Kempton & Leng, 1979). It is important, however, not to increase intake to such an extent that retention time in the rumen and/or intestine is too short to thoroughly ferment and digest the substrate, but to determine the optimum balance between an increased voluntary feed intake, rumen fermentation rate and N status of the animal.

It is not only important to determine the effect of protein and/or urea supplementation on voluntary feed intake, but also on the end products of fermentation and digestion. In this

regard, special attention must be given to the fermentation process in the rumen, specifically to VFA concentrations and ratios, as well as MP synthesis. Higher levels of NH_3 in the rumen will cause increases in rumen pH, which in turn will have an influence on the composition of the rumen microbial population. An altered rumen microbial population will inevitably lead to a change in fermentation products. Therefore, it is necessary to determine the effect of N supplementation and an increased voluntary feed intake on the provision of fermentation products in the lower digestive tract.

Microbial protein produced in the reticulo-rumen is the primary source of amino acids available to ruminants fed roughage diets. In this regard, Hume *et al.* (1970) suggested that the maximum theoretical MP yield is about 17 g/100g OM digested in the rumen. Various factors, such as N, digestible energy and C-skeleton availability to the rumen microbes, as well as N to sulphur (S) ratio (optimum ratio being about 10:1; Doyle *et al.*, 1982) will have a defined influence on MP production. In order to provide all the amino acids in their specific required amounts to meet the requirements of the animal, the microbes should therefore produce MP at the optimum level of efficiency.

Furthermore, evidence from several workers suggest that the effects of N intake in the ruminant be mediated through the microbial population in the rumen (Hume *et al.*, 1970). The highly significant relationship between direct counts of rumen bacteria and nitrogen intake supports their findings, because the direct count of rumen bacteria reflects the functional capacity of the rumen (Moir & Harris, 1962).

Since RDP supplementation has such a remarkable influence on the fermentation and digestive processes of the ruminant animal, these possible changes have to be considered when expressing the optimum RDP requirement. Therefore, the amount of RDP required to optimise animal performance, will be expressed as a percentage of digestible organic matter intake (DOMI). This specific criterion, DOMI, represents an integrated assessment of treatment effects on forage intake and digestion. Thus, expressing the desired amount of RDP as a percentage of DOMI accentuates the fact that the RDP required to maximise DOMI will vary with the inherent digestibility of the forage.



A fair amount of work has been done on sheep, concerning the use of natural protein and NPN supplements to low-quality roughages, and the benefits of such supplementation strategies have been highlighted. However, in many of these studies the inclusion levels of urea and/or protein have been chosen arbitrarily and most of these research focused on the effects of providing these sources as individual supplements, which illustrated that natural protein is a more efficient RDP supplement than NPN. Furthermore, many of these natural protein supplements contained certain amounts of rumen undegradable protein, which would probably have favoured the protein supplements in comparison to NH_3 . Since urea is much less expensive than natural protein per unit N and cellulolytic bacteria can grow with NH_3 as the sole N source (Russell *et al.*, 1992), it is necessary to determine to what extent urea can replace natural protein in RDP supplements without sacrificing animal performance. Information concerning the effects of RDP vs urea supplements on fermentation and digestion characteristics of sheep consuming low-quality forages seems to be limited. Therefore, further research in this field is of utmost importance.

The purpose of this study was to determine the supplemental RDP requirement and ideal NPN to natural RDP ratio to maximise DOMI, in Dohne Merino sheep fed a low-quality, high roughage diet (3.2% CP; 74.2% NDF). In Chapter 2, the supplemental protein requirement was determined using a natural protein supplement (calcium caseinate) at different inclusion levels. This optimum RDP requirement was further investigated in a study reported in Chapter 3, where different amounts of natural protein were replaced by NPN on an isonitrogenous basis.

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CHAPTER 2

Effect of different Rumen Degradable Protein levels on the Utilisation of Wheat Straw by Dohne Merino Wethers

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Abstract

A 5 x 5 Latin square was conducted with ruminally and duodenally cannulated Dohne Merino wethers to determine the effect of different rumen degradable protein (RDP) levels on forage intake, fermentation characteristics, digestion and nutrient flow in wethers consuming wheat straw. The wethers had *ad libitum* access to water and wheat straw (3.2% CP; 74.2% NDF) which was fed twice daily, immediately after intraruminal infusion of the supplements at 07h00 and 19h00. The supplemental RDP (calcium caseinate: 90% CP; 100% rumen degradable) levels were: 0, 40, 80, 120 and 160 g/d. Each period consisted of 14 days of adaptation and 6 days of sampling. Forage and total organic matter (OM) intakes tended to increase quadratically ($P \leq .15$) with higher supplemental RDP levels. Effects of treatment on rumen and total tract digestion were minimal. Digestible OM intake (DOMI) displayed a quadratic increase with elevated amounts of RDP ($P < .01$), and was maximised at 3.15 g RDP/kg BW^{.75} or 11.6% of DOM. Microbial nitrogen (MN) flow to the duodenum and microbial efficiency increased quadratically ($P \leq .04$) and fluid dilution rate tended to increase in a quadratic manner ($P = .15$) with increased RDP supplementation. Ruminal ammonia nitrogen (NH₃-N) and total volatile fatty acid (VFA) concentrations increased linearly ($P \leq .07$) while rumen pH exhibited a variable response to increased RDP levels (cubic; $P = .08$). In conclusion, increasing RDP supplementation to Dohne Merino wethers consuming wheat straw generally enhanced forage utilisation and digestion.

Key words: Digestible organic matter intake; Wheat straw; Rumen degradable protein; Sheep

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Introduction

Low-quality crop residues or dry grass forages are the primary sources of energy available to grazing ruminants for a considerable time of the year. These forages are unpalatable, have a high cell wall constituent, low digestibility and are deficient in N, with a subsequent low level of rumen microbial activity. The efficiency of the rumen digestive system is dependent upon the maintenance of a functional rumen microbial population and the level of activity of this population (Moir & Harris, 1962). A more active and efficient microbial population may improve the energy status of grazing ruminants by promoting greater intake and/or digestion, with a subsequent higher nutrient flow to the duodenum, and possibly via improving the efficiency of utilisation of metabolisable energy (ME). This implies that the energy, primarily cellulose, contained in the fibre component of these low-quality roughages will be more readily available to the ruminant animal.

The pre-dominant nutritional requirements of the rumen microbes are N and energy (Henning, 1990), but generally N is considered to be first limiting (Kempton & Leng, 1979; Freeman *et al.*, 1992; Mawuenyegah *et al.*, 1997). Evidence that supports this view, states that protein supplementation to ruminants grazing low-quality forage has increased DM intake as a result of an increased rate of digestion and/or passage to the small intestine (Church & Santos, 1981; DelCurto *et al.*, 1990; Matejovsky & Sanson, 1995). In contrast, energy supplementation has in general been ineffective in improving the energy status of ruminants consuming low-quality roughages (DelCurto *et al.*, 1990).

Since protein supplementation is expensive, it is necessary to determine the amount of rumen degradable protein (RDP) required to maximise low-quality forage intake and digestibility, as well as microbial protein (MP) synthesis in order to optimise animal production. Köster *et al.* (1996) studied the RDP requirements of beef cattle consuming low-quality forage, and concluded that supplementing 4.01 g RDP/kg BW^{0.75} or 11.1% of digestible organic matter (DOM) sufficiently enhanced rumen fermentation and digestion characteristics to maximise digestible organic matter intake (DOMI). This raised the question whether sheep would respond in a similar manner when consuming low-quality wheat straw. Therefore, the objective of this study was to determine the supplemental RDP requirement needed by sheep

to maximise DOMI of low-quality wheat straw. Associated effects on ruminal fermentation characteristics were also measured.

Material and Methods

Five Dohne Merino wethers with an average live weight of 60 kg, fitted with rumen fistulas and duodenal cannulas were used in a 5 x 5 Latin square to evaluate the effects of increasing RDP levels on forage intake and utilisation characteristics. Animals were housed individually in 1 x 2 m metabolism cages and had *ad libitum* access to wheat straw (Table 1) and water. The wheat straw was chopped into 50 mm pieces with a hammermill and offered at 140% of the average intake of the previous five days for each individual animal. Experimental treatments provided the following supplemental RDP levels: (1) control, 0 g RDP/d, (2) 40 g RDP/d, (3) 80 g RDP/d, (4) 120 g RDP/d and (5) 160 g RDP/d. The RDP in the form of calcium caseinate (90% CP; 100% rumen degradable) was dissolved in 1.2 L water, divided into two equal portions and administered intraruminally at 07h00 and 19h00 just before feeding the forage. To prevent possible trace and macro mineral deficiencies, a mineral premix (26% NaCl, 16% Ca, 8% P, .004% I₂, .001% Co, .25% Mn and .22% Zn) was formulated based on the mineral content of wheat straw and was infused intraruminally with the morning supplements at 19.05 g/wether/d.

Sampling

The trial consisted of 5 periods of 20 d each. Animals were allowed to adapt to the diet and their specific supplements for the first 14 d of each period. During the following 4 d, voluntary feed intake was measured and representative wheat straw samples were taken at each feeding time. Orts were removed before the morning feeding and kept as individual samples for every animal within each period. From d 16 through 18, duodenal and faecal samples were taken at 6 h intervals, starting at 24h00. Sampling time was advanced 2 h each day to obtain samples on every even hour of a 24 h period. On d 19 the rumen of each sheep was completely emptied at 06h00, just before feeding and infusion of supplements, and again at 15h00, to obtain a representative sample of rumen contents. The content was weighed, mixed and a 1 kg sample was taken and frozen immediately.

At 06h00 on d 20, 15 mL of Cr-EDTA, (360 mg Cr/15 mL; Uden *et al.*, 1980) was infused into various ruminal sites for determination of fluid dilution rate. Sixty mL of ruminal fluid was extracted at 0, 3, 6, 9, 12 and 24 h and the pH of these samples were measured at the time of extraction from the rumen. Ruminal fluid samples obtained at 0, 3, 6, 9 and 12 h were used to prepare samples for determination of both volatile fatty acids (VFA`s) and ammonia nitrogen (NH₃-N) concentrations. A mixture of 9 mL rumen fluid and 1 mL of a 10% (m/v) NaOH preservative solution was analysed for VFA`s (Cottyn & Bouche, 1968) and 8 mL of a .1 N HCl solution was added to 2 mL rumen fluid to determine rumen NH₃-N concentrations (Köster *et al.*, 1996). All samples were frozen immediately to be analysed later.

A specially developed, but very simple device was used for the ruminal fluid extractions. This apparatus consisted of the casing of a 5 mL syringe, inserted into an *in situ* degradation bag with 50 μ diameter pores. The syringe had 25 pores with a diameter of 3 mm each and the open end was blocked with a rubber stopper. A 20 cm long rubber tube was connected to the nozzle of the syringe and passed through the cork of the rumen fistula. The tube was connected externally to a small tap which could be attached to a 60 mL syringe to extract ruminal fluid. Merits for using this apparatus are that the tap is only opened during extraction and thus conditions in the rumen are kept anaerobic and constant. In addition ruminal fluid obtained in this manner is clean and contains no large feed particles.

Laboratory Analyses

Faecal samples were dried in a normal drying oven for 96 h at 50°C. Feed, orts and faecal samples were ground to pass a 1 mm screen with a Scientec Hammer Mill (Peter Rassloff, Instruments & Services (PTY) Ltd.). Faecal and orts samples were pooled for each wether within each period, while the 5 dietary samples for each period were pooled to form 1 representative feed sample. These samples were analysed for N, NDF and acid insoluble ash (AIA). Acid insoluble ash (Cottyn & Bouche, 1968; .2 N HCl procedure) was used as a marker to calculate digestibility coefficients. All N and NDF analysis were done according to the methods of the AOAC (1984) and N was measured with a Leco Auto Analyser (Model FP 428). For determination of DM, feed, orts and faecal samples were dried at 100°C in a

convection oven. Samples were ashed for 5 h at 500°C to determine organic matter (OM) concentrations (AOAC, 1984).

Duodenal samples were lyophilised and then pooled for each wether within each period. These pooled samples were finely ground with a Scientific Apparatus sample mill (Arthur H. Thomas Co., Philadelphia, PA) and analysed for N, purine concentration (Zinn & Owens, 1986), NDF and AIA. For determination of NH₃-N concentrations in the duodenum, samples were reconstituted to 3% DM in a .1 N HCl solution, mixed and centrifuged at 20 000 x g for 20 min (Hannah *et al.*, 1991). The supernatant was then poured off and analysed for NH₃-N according to the phenol hypochlorite procedure described by Broderick & Kang (1980).

Ruminal fluid samples for determination of VFA's and NH₃-N were thawed at room temperature. Ammonia N samples were analysed according to the aforementioned procedure described by Broderick & Kang (1980) and VFA concentrations were measured by gas chromatography. Chromium concentration in ruminal fluid samples were diluted (1:2 dilution) and Cr concentration measured with a radial emission, inductively coupled plasma spectrometer (Liberty Series II). Fluid dilution rate was determined by regressing the natural logarithm of Cr concentration on sampling time (Warner & Stacy, 1968).

Rumen bacteria were isolated by adding a saline solution (9 g NaCl/L; 500 mL/kg rumen content) to the rumen contents and blending the mixture for approximately 1 min. The samples were then strained through 2 layers of cheesecloth and centrifuged at 1000 x g for 10 min to remove feed particles and protozoa. The supernatant was poured off and centrifuged at 20 000 x g for 20 min to isolate the bacteria. Once again the supernatant was poured off, the pellets were washed with saline solution (9 g NaCl/L), duplicates were combined and re-centrifuged at 20 000 x g for 20 min. Isolated bacteria were frozen, lyophilised and analysed for purine concentration.

Statistical Analysis

Intake, digestibility and flow data were analysed as a 5 x 5 Latin square, using the GLM procedure of SAS (1994), with effects for animal, period and treatment. Treatment sums of squares were partitioned into linear, quadratic and cubic effects of RDP level with orthogonal

polynomials. Since DOMI is a comprehensive concept which integrates treatment effects on intake, fermentation and digestion characteristics, RDP requirement was expressed as the amount required to maximise DOMI. In order to obtain this value, the first derivative of the quadratic regression of DOMI on RDP intake was determined, set equal to zero and solved for x . The resulting value was the amount of RDP required to maximise DOMI. This value was inserted into the original regression equation and the equation solved to determine maximum DOMI. Required RDP intake was then expressed as a percentage of this calculated maximum DOMI. In addition RDP intake required for maximum DOMI was determined using a single slope, broken-line model (Robbins, 1986) with the NLIN procedure of SAS (1994).

Rumen pH, VFA and $\text{NH}_3\text{-N}$ concentrations were analysed as a Latin square split-plot design. Whole-plot sources of variation were animal, period and treatment, and the subplot sources were time and treatment x time interaction. Whole-plot effects were tested by animal x period x treatment and residual error was used to test the subplot effects. Treatment sums of squares were partitioned into linear, quadratic and cubic contrasts.

Results and Discussion

From Table 1 it is evident that wheat straw is low in nutritional content. The high fibre and low CP content results in a low digestibility, rendering wheat straw unsuitable to maintain body weight or meet the higher nutrient requirements of producing sheep (Aitchinson, 1988). Meissner *et al.* (1991) stated that material with a NDF content above 55 - 60% depresses voluntary intake. In addition, Thornton & Minson (1972) reported that the passage rate of feed through the rumen is largely determined by characteristics of the diet, pre-dominantly the fibre content, rather than by physiological factors of the animal. Therefore, it can be assumed that wheat straw will have a longer rumen retention time than higher quality forages, which in turn will impair voluntary feed intake and thus animal performance. However, since wheat straw is the sole feed source available to grazing sheep at certain times of the year, its utilisation as a low cost source of energy has to be optimised.

Table 1. Chemical composition of wheat straw and calcium caseinate on a DM basis

Item	Wheat Straw (%)	Casein (%)
DM	93.5	89.1
Ash	4.3	2.9
OM	95.7	97.1
CP	3.2	90.0
NDF	74.2	0
ADF	53.6	0
AIA	0.66	0
Fat	0.86	0.23

Intake and Digestibility of Forage and N

Forage intake responses to supplemental protein are usually observed when the CP content of forages is less than 6 - 8% (Kartchner, 1980; Matejovsky & Sanson, 1995). The availability of CP to the rumen microbial population or enzyme activity in the lower gut will vary according to the digestibility of the forage. Therefore, both digestible CP content and forage digestibility must be considered when predicting intake responses to supplemental protein. Other supplement characteristics such as energy source, protein degradation rate, and synchronisation of protein degradation and fermentation rate of the forage may also influence the extent to which CP concentration of the supplement stimulates intake (Hannah *et al.*, 1991).

The effect of increasing proportions of supplemental RDP on intake and digestibility characteristics are presented in Table 2. In the current study, intake of forage OM, total OM and forage N tended to increase in a quadratic manner ($P \leq .15$) with increasing proportions of supplemental RDP. The largest incremental increase was observed between the control (0 g RDP/d) and 40 g RDP/d treatment. Thereafter intake still increased, but at a diminishing rate. These results confirm earlier findings that protein supplementation to ruminants consuming low-quality forages can enhance forage intake (Church & Santos, 1981; DeICurto *et al.*, 1990; Matejovsky & Sanson, 1995). It is however important to acknowledge the fact that in the present study the protein supplement was 100% rumen degradable. The observed response can thus be attributed to treatment effects on the rumen microbial population. The increased forage OM intake response as a result of supplemental RDP confirmed that the wheat straw (3.2% CP) was deficient in N and therefore RDP supplementation effectively

enhanced the utilisation of this low-quality roughage. Köster *et al.* (1996) showed similar responses in intake parameters when incremental levels of RDP were provided to beef cows fed low-quality prairie hay (1.94% CP).

Table 2. Effect of increasing proportions of supplemental RDP on intake, digestibility and duodenal flow in Dohne Merino wethers fed wheat straw

Item	RDP (g/d)					SEM ¹	Contrasts ²		
	0	40	80	120	160		L	Q	C
OM Intake	g/kg BW ^{.75}								
Forage	41.12	52.03	53.80	57.51	56.92	3.63	<.01	.15	.68
Casein	0	2.09	4.15	6.05	8.21				
Total	41.12	54.12	57.94	63.56	65.13	3.41	<.01	.12	.64
Digestible OM Intake	14.15	25.00	26.98	27.67	27.10	1.43	<.01	<.01	.11
N Intake	g/kg BW ^{.75}								
Forage	.21	.27	.28	.30	.30	.02	<.01	.15	.68
Casein	0	.31	.62	.90	1.22				
Total	.21	.58	.90	1.20	1.52	.03	<.01	.28	.42
Ruminal Digestibility	%								
Apparent OM	33.83	43.32	37.95	37.72	37.40	3.94	.90	.35	.26
True OM	34.59	46.85	42.09	41.98	41.62	4.07	.49	.21	.21
NDF	37.21	49.65	46.38	41.96	46.57	2.98	.27	.18	.02
Apparent N	-26.48	5.35	15.32	18.50	36.53	13.87	<.01	.52	.42
Duodenal Flow	g/d								
Total N	7.13	12.36	16.41	17.11	15.25	1.62	<.01	.02	.69
Microbial N	4.66	8.31	10.50	10.44	9.16	1.20	.05	.04	.92
Ammonia N	.12	.52	1.12	1.51	1.77	.12	<.01	.39	.32
NMNAN ³	1.81	3.19	4.67	5.48	5.18	.64	<.01	.16	.49
Fluid Dilution Rate	%								
	6.53	6.74	7.41	7.23	6.08	.54	.55	.15	.34
Microbial Efficiency	g N synthesised/kg OM truly digested								
	8.80	11.65	16.12	15.62	13.38	1.51	.05	.03	.42
Total Tract Digestibility	%								
OM	34.40	46.38	43.97	43.95	42.15	5.58	.47	.25	.49
NDF	37.97	52.44	45.30	49.54	53.41	5.76	.15	.66	.27
N	-31.64	24.69	40.66	53.11	60.17	7.71	<.01	<.01	.11

¹Standard error of the mean (n=5).

²L = linear, Q = quadratic, C = cubic.

³Nonmicrobial-nonammonia N.

In a review of research on protein supplementation, Owens *et al.* (1991) reported that improved animal performance, as a result of protein supplementation, was mediated through either an increased DOMI and/or enhanced efficiency of ME utilisation. These authors stated that despite the relative importance of efficiency of ME utilisation, most research indicated

that an increased DOMI was the primary cause of enhanced animal performance as a result of protein supplementation. In addition, DOMI will inevitably vary with digestibility of the forage, rendering the expression of the RDP requirement as a percentage of DOM much more appropriate, since it will vary in the same manner as digestibility. Therefore, striving to maximise DOMI and expressing supplemental RDP requirement as a percentage of DOM should be the most appropriate response criterion for determining RDP requirements on roughage-based diets.

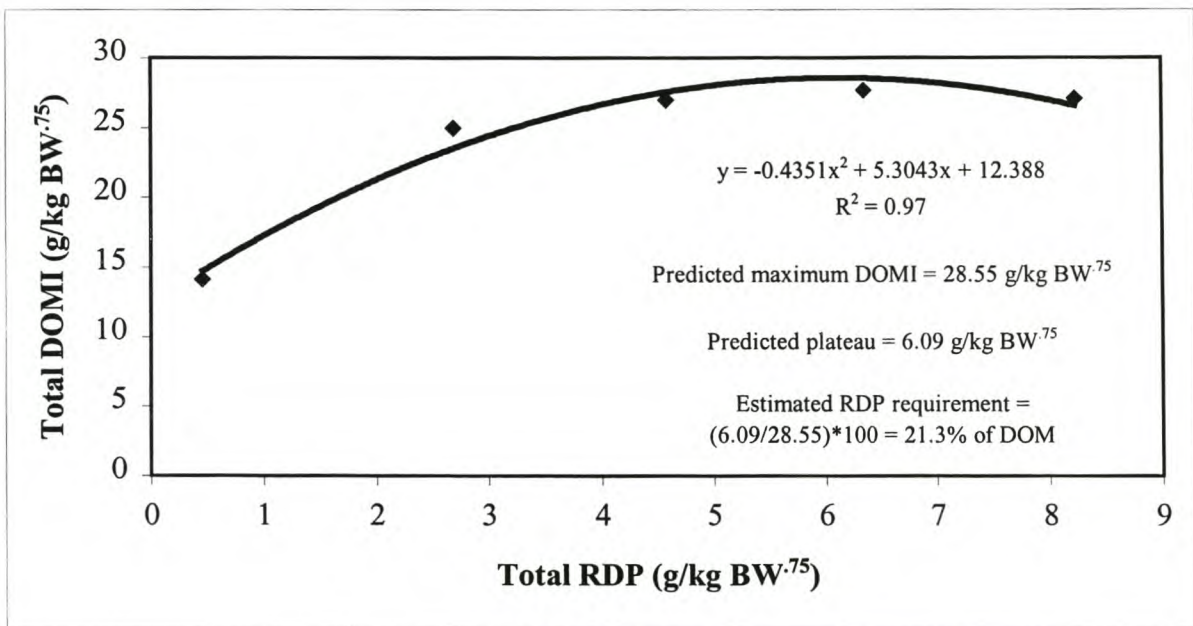


Figure 1. RDP required to maximise DOMI, estimated by calculating the first derivative of the quadratic regression equation

Digestible OM intake displayed a quadratic response ($P < .01$) to increasing supplemental RDP levels. The largest incremental increase was observed with the first addition of supplemental RDP (40 g/d). Thereafter intake generally increased but at a diminishing rate. Determining the amount of RDP required to maximise DOMI from the first derivative of the quadratic regression equation for DOMI, resulted in a calculated RDP requirement of 21.3% of DOM (6.09 g RDP/kg BW^{0.75}, Figure 1). Generally the quadratic regression procedure yields larger values, because it predicts requirements where maximum response is obtained (Baker, 1986). Therefore the single slope, broken-line model (Robbins, 1986) was also fitted on the data. The broken-line model predicted the estimated total DOMI as 27.2 g/kg BW^{0.75},

with an associated RDP requirement of 11.6% of DOM (3.15 g RDP/kg BW^{.75}, Figure 2). Considering the high cost of protein supplementation and the reduced magnitude of incremental improvements in DOMI as maximum response is approached, the broken-line model renders itself to be the more cost effective approach (Baker, 1986; Robbins, 1986). Köster *et al.* (1996) also used the single slope, broken-line model and predicted that the RDP requirements of beef cows consuming low-quality forage (1.94% CP) was 11.1% of DOM.

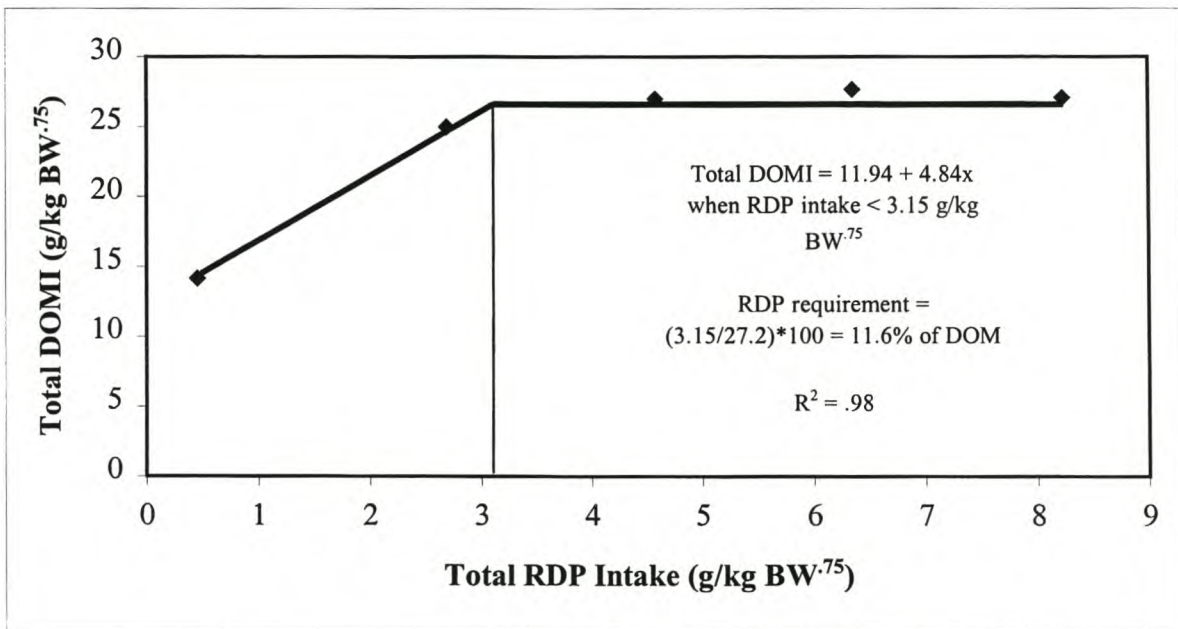


Figure 2. RDP required to maximise DOMI, using a single slope, broken-line model

Neither apparent or true ruminal OM digestibility, or total tract OM digestibility were affected by elevated levels of supplemental RDP ($P \geq .21$). Ruminal NDF digestibility displayed a variable cubic response ($P = .02$) with increasing RDP levels, while total tract NDF digestibility tended to increase at higher RDP supplementation levels ($P = .15$). However, all OM and NDF digestibility parameters displayed a minimum value at the control treatment, with a concomitant biggest incremental increase between the control treatment and 40 g RDP supplementation. At higher RDP supplementation levels digestibility parameters stayed relatively constant. It is concluded that wheat straw alone is deficient in N and unable to maintain optimum digestive processes. These results coincide with the findings of Church & Santos (1981) who suggested that, other than for CP, an increased forage intake is not necessarily accompanied by increases in digestibility. It is worth mentioning that time spent

ruminating per 100g NDF intake is considerably longer at low than high levels of feed intake (Mawuenyegah *et al.*, 1997). Although treatment enhanced voluntary feed intake in the present study (from a minimum of 43 g/kg BW^{.75} for the control treatment to a maximum of 60 g/kg BW^{.75} for the 120 g RDP/d treatment), intakes were still relatively low compared to higher quality forages (94 g/kg BW^{.75}), as reported by Blaxter *et al.* (1961). It can thus be assumed that the results of Semiadi *et al.* (1994), which indicates that a higher frequency of rumination activity could be expected to lead to a more efficient rate of particle breakdown, a faster rate of rumen content turnover and an increased saliva production and therefore an enhanced DM intake, would be applicable in the present study.

In contrast to OM and NDF digestibility, apparent ruminal N digestibility increased linearly ($P < .01$) and total tract N digestibility increased quadratically ($P < .01$) with increasing proportions of supplemental RDP. Since calcium caseinate is highly soluble, a reduced rumen retention time, resulting from higher intakes, should not affect its degradation. The enhanced N digestibilities at higher RDP levels observed in the present study, can thus probably be attributed to an increased digestibility of calcium caseinate N and not necessarily forage N. The negative total tract N digestibility value in the present study for the control treatment and subsequent increased N digestibility with increasing levels of supplemental RDP is in agreement with the results of Church & Santos (1981). In corroboration Bunting *et al.* (1989) reported a greater abomasal N flow than N intake in calves fed a low-protein diet. This type of response can be attributed to the contribution of endogenous N.

Nutrient Flow to the Duodenum

Thornton & Minson (1972) determined that DM intake had an inverse relationship with retention time, which Colucci *et al.* (1982) and Gregory *et al.* (1985) verified by stating that passage rate of digesta through the entire digestive tract increased with higher DM intakes. In addition Thornton & Minson (1973) observed that rumen OM retention time was highly correlated with daily intake of digestible OM in sheep fed grasses and legumes. An increased OM intake, as observed in the present study, will result in a greater substrate flow to the rumen and subsequently improve microbial growth and increase efficiency of MP synthesis, resulting in a higher flow of microbial nitrogen (MN) to the duodenum (Sniffen & Robinson, 1987; Djouvinov & Todorov, 1994).

Fluid dilution rate (Table 2) showed a weak quadratic improvement ($P = .15$) with incremental increases in supplemental RDP levels, and reached a maximum of 7.4%/h at 80 g RDP/d. The response in fluid dilution rate was similar to that of feed intake. A higher feed intake will lead to an enhanced saliva flow and water consumption (Hannah, 1991), which will result in an elevated liquid dilution rate (Robinson *et al.*, 1985). Furthermore, Van Soest (1982) suggested that an increased intake results in a greater flow of particles from the rumen that are at an earlier stage of digestion, with more attached microbes. This is verified in the present study, by the observed increase in MN flow to the duodenum at higher OM intakes.

From Table 2 it is apparent that both total N and MN flow to the duodenum displayed a quadratic increase ($P \leq .04$) with increasing levels of supplemental RDP. Duodenal MN flow reached a maximum of 10.5 g/d at a RDP level of 80 g/d and showed no further response as RDP levels increased. Similar to the response observed with DOMI, the biggest incremental response in MN flow to the duodenum was between 0 and 40 g RDP/day, after which further incremental increases declined. This response indicates a definite N deficiency in the control treatment. Although 80% of rumen microbes can grow with NH_3 as their sole N source (Baldwin & Allison, 1983), the increased MN flow to the duodenum can not be solely attributable to an increased ruminal NH_3 -N concentration. Motivation for this statement is that various studies have indicated the positive effects of natural protein supplementation to rumen micro-organisms, with a concomitant stimulation of fibre digestion and microbial growth rate and yield (Cotta & Russell, 1982; Merry *et al.*, 1990; McAllan, 1991). It is difficult to distinguish whether these benefits stem from the supply of pre-formed amino acids to the rumen micro-organisms or to the escape of some of the dietary protein to the abomasum. However, in the present study the protein supplement was 100% rumen degradable. Thus, the observed stimulatory effect on the rumen microbial population can be partially attributed to an increased NH_3 -N concentration, as well as the provision of amino acids and peptides, which can be directly incorporated into MP with a subsequent increase in efficiency of microbial synthesis. Furthermore Bunting *et al.* (1987) controversially stated that the dietary protein level does not affect the percentage of bacterial N synthesised from ruminal NH_3 -N. In their study, however, the supplemental protein was not all rumen degradable and may have provided a significant amount of undegradable, essential amino acids in the lower gastro-intestinal tract. Conditions as such will improve the N status of the

animal, without significantly affecting rumen $\text{NH}_3\text{-N}$ concentrations. The observed increase in forage OM intake at higher supplemental RDP levels, which may have enhanced N flow (Van Soest, 1982; Robinson *et al.*, 1985; Merchen *et al.*, 1986), could also have contributed to the elevated levels of MN flow to the duodenum.

As expected, higher levels of RDP supplementation resulted in a linearly increased ($P < .01$) $\text{NH}_3\text{-N}$ flow to the duodenum (Table 2). Expressed as a percentage of total N flow to the duodenum, $\text{NH}_3\text{-N}$ flow increased from 1.7% with the control treatment to 11.6% at 160 g RDP/day, which corresponds well with the 3 - 9% reported by Tamminga *et al.* (1979) and Varga & Prigge (1982). Since calcium caseinate is 100% rumen degradable and highly soluble, NH_3 was probably produced in the rumen at a faster rate than it could be utilised for MP synthesis. In addition, higher levels of RDP provided more substrate to the proteolytic bacteria with a subsequent enhanced protein degradation and NH_3 release, increased fermentation activity and resultant increase in intake and ruminal turnover rate. The direct consequence of the above mentioned factors would be a higher outflow of nutrients, including NH_3 , to the duodenum.

Nonmicrobial-nonammonia N (NMNAN) consists of epithelial cells derived from the respiratory tract, mouth and oesophagus, keratinised epithelial tissue from the rumen wall (Ørskov *et al.*, 1986) and rumen undegradable dietary protein. The linear increase ($P < .01$) of NMNAN flow to the duodenum (Table 2) can be attributed to the increased OM intake and concomitant increase in passage rate, resulting in more epithelial tissue being sloughed from the intestinal wall. In addition, a higher OM intake and reduced rumen retention time may lead to a higher proportion of dietary protein not being degraded in the rumen, with a consequent increased NMNAN flow to the duodenum. However, Firkens *et al.* (1986) reported that intake has no effect on NMNAN flow to the duodenum.

Rumen Fermentation Characteristics

The protein synthesising capability of the rumen microbial population was highlighted when Virtanen (1966) illustrated that the protein requirement of an average producing cow could be met by the rumen microbes with urea as the only dietary N source. Since then various research studies have verified the importance of the rumen microbial population in both MP

synthesis and carbohydrate digestion. Sniffen & Robinson (1987) stated that the ruminant receives 40 to 80% of its daily amino acid requirements from MP flowing to the small intestine.

In the present study microbial efficiency exhibited a quadratic response to treatment ($P = .03$), increasing with elevated proportions of RDP to reach a maximum of 16.1 g MN synthesised per kg OM truly digested at 80 g RDP/day (Table 2). These values are slightly lower than the average microbial efficiency of 19.3 g MN/kg OM observed by Czerkawski (1978) to be truly fermented in the rumen. Hogan & Weston (1967) calculated microbial efficiency to be 24 - 25 g MN/kg OM truly digested, for high (19.8%) and low protein (7.8%) diets respectively. A possible explanation for the slightly lower microbial efficiencies observed in the present study is that the wheat straw diet provided less fermentable energy to the microbes than did the higher quality diets in the above mentioned studies, resulting in slow microbial growth rates (Owens & Goetsch, 1988). However, there is little variation in the values, which illustrates the potential of supplemental RDP to enhance rumen fermentation and thus improve the utilisation of the relatively indigestible fibrous energy sources.

In addition, the response in microbial efficiency can possibly be mediated via the higher feed intakes associated with higher levels of RDP (Köster *et al.*, 1996), resulting in increased liquid dilution rates (Robinson *et al.*, 1985; Firkens *et al.*, 1986; Merchen *et al.*, 1986; Volden, 1999). An increased dilution rate is usually associated with an enhanced particulate passage rate (Evans, 1981) and thus a reduced retention time of bacteria in the rumen, since a greater proportion of total bacterial cells leaving the rumen is associated with the particulate matter rather than with the liquid phase (Faichney, 1980). Therefore, the maintenance energy requirement of the rumen population is decreased and more energy is available for growth (Isaacson *et al.*, 1975), resulting in a larger rumen microbial population with an increased activity, which is manifested in the observed increase in microbial efficiency in the present study.

Previous research has shown that pH affects microbial growth rate, microbial efficiency and microbial end-products (Russell *et al.*, 1979; Russell & Dombrowski, 1980; Erfle *et al.*, 1982). Results of Hoover *et al.* (1984) suggested that pH is more important in controlling

microbial activity than dilution rate and that both low (5.5) and high (7.5) pH levels severely depress fibre digestion. According to Table 3, pH responded variably (cubic; $P = .08$) to increased RDP supplementary levels. The pH was, however, relatively constant and only varied between a minimum of 6.77 at 40 g RDP/d and a maximum of 6.89 at 120 g RDP/d. These values are well above 6.2 where below cellulolytic activity may be depressed (Williams *et al.*, 1984) and close to 6.5 which is regarded as the optimum for fibre and OM digestion (Hoover *et al.*, 1984). The relatively high pH values were probably a result of the high levels of $\text{NH}_3\text{-N}$ from the supplemental RDP as well as re-circulation of NH_3 and bicarbonate via an increased parotid saliva secretion associated with higher consumption of more fibrous diets (Doyle *et al.*, 1982). It also indicates a slow carbohydrate fermentation rate as a result of the high fibre content of the wheat straw and a subsequent slow release of VFA's.

Proteolytic bacteria in the rumen degrade the RDP to NH_3 , amino acids and peptides which are utilised by the rumen microbial population to produce MP. Therefore, as expected, $\text{NH}_3\text{-N}$ increased linearly ($P < .01$) with increasing proportions of supplemental RDP (Table 3). Since casein is 100% rumen degradable and highly soluble, NH_3 was released at a fast rate in the rumen shortly after supplement infusion, resulting in elevated $\text{NH}_3\text{-N}$ levels. Despite these relatively high levels of rumen $\text{NH}_3\text{-N}$, the maximum rumen pH in the present study was $6.89 (\pm .02)$. Therefore, it may be expected that most of the NH_3 would have been available for MP synthesis, since passive absorption of NH_3 from the rumen into the bloodstream is facilitated by a pH greater than 7 (Chalmers *et al.*, 1976). According to Bryant & Robinson (1963) approximately 80% of rumen microbes can grow with NH_3 as their sole source of N, which is supported by the response in MN flow to the duodenum observed in the current study. When N availability in the rumen is limited, bacterial action shifts from synthesis of MP to synthesis of intracellular polysaccharides (McAllan & Smith, 1977). Such a scenario is not ideal, because protein supplementation is more expensive than energy supplementation and therefore it is important to maximise the protein synthesising ability of the rumen microbes.

Firkens *et al.* (1986) found a positive relationship ($r = .75$) between true ruminal OM digestion and ruminal $\text{NH}_3\text{-N}$ concentration in steers. Mawuenyegah *et al.* (1997) is of opinion that the rate of digestion of the plant cell wall is dependent on sufficient N and

readily available energy for rumen microbial growth, their access to the cell wall carbohydrates and particle size reduction. Satter & Slyter (1974) determined that when NH₃-N levels in the rumen fluid exceed 50 mg/L, NH₃-N starts to accumulate in the rumen and has no further beneficial effect on MP production. These authors also noted that excessively high levels of NH₃, up to 800 mg NH₃-N/L, did not inhibit microbial growth. Excess NH₃-N will, however, increase the maintenance requirement of the animal in terms of the additional energy cost required for excretion of the abundant NH₃-N. It was concluded that an NH₃-N concentration of 20 - 50 mg/L is sufficient to allow maximum growth of rumen microbes (Slyter *et al.*, 1979). Ruminal NH₃-N concentrations exceeded these requirements in all treatments, except for the control.

To the contrary, Perdok *et al.* (1988) reported the NH₃-N requirement for maximum voluntary intake of low-quality forage as 200 mg/L rumen fluid. This concentration was only exceeded in treatment 5 (160 g RDP/d), but forage OM intake did not differ for any of treatments 2 - 5 (Table 2). In addition, DOMI increased quadratically ($P < .01$) and reached a plateau with increasing proportions of RDP. Therefore, it is concluded that the corresponding NH₃-N requirement for maximum DOMI was met at ± 88 mg/L (3.15 g RDP/kg BW^{0.75}), even though it is lower than suggested by Perdok *et al.* (1988).

Table 3. Effect of increasing proportions of RDP on pH, NH₃-N and VFA concentrations in the rumen of Dohne Merino wethers fed wheat straw

Item	RDP (g/d)					SEM ¹	Contrasts ²		
	0	40	80	120	160		L	Q	C
PH	6.83	6.77	6.87	6.89	6.81	.02	.53	.40	.08
NH ₃ -N (mg/L)	16.69	72.82	136.99	179.37	218.98	8.89	<.01	.36	.83
Total Volatile Fatty Acids (mM)	53.47	62.08	55.40	60.38	62.43	1.21	.07	.95	.12
	Mol/100 mol								
Acetate	76.29	75.58	73.61	72.39	70.02	.29	<.01	.43	.98
Propionate	19.28	19.75	19.82	19.34	20.49	.18	.27	.66	.25
Butyrate	4.43	4.12	5.57	5.90	5.77	.22	.02	.63	.19
Isobutyrate	0	.33	.66	1.42	1.27	.21	<.01	.38	.39
Valerate	0	0	0	.96	1.84	.28	<.01	.06	.93
Isovalerate	0	.03	.34	.54	.61	.11	<.01	.96	.44
Acetate:Propionate	4.00	3.86	3.74	3.74	3.43	.03	<.01	.62	.38

¹Standard error of the mean (n=5)

²L = linear, Q = quadratic, C = cubic

Total VFA concentration increased linearly with increasing increments of supplemental RDP ($P = .07$). This is in agreement with the linear increase ($P < .01$) in $\text{NH}_3\text{-N}$ concentration with increasing RDP supplements, which may lead to the conclusion that rumen $\text{NH}_3\text{-N}$ levels without supplementation were insufficient to support maximum fermentation. However, Slyter *et al.* (1979) observed that $\text{NH}_3\text{-N}$ levels above 45 mg/L had no significant effect on either the VFA concentration or N retention. In addition the increased total diet intake, when greater amounts of RDP were supplemented, would also stimulate a higher total VFA production, due to an increased quantity of substrate available for fermentation (Staples *et al.*, 1984). In his study, Volden (1999) also reported a reduced total VFA concentration due to a decreased feed intake.

From Table 3 it is evident that the acetate proportion and the acetate:propionate ratio decreased linearly ($P < .01$) with proportional increases in RDP level, while molar percentage of propionate showed no response to treatment (cubic; $P = .27$). The reduction in the acetate:propionate ratio can thus be attributed to a reduced acetate production rather than an increased propionate synthesis. Butyrate, isobutyrate, valerate and isovalerate proportions increased linearly with elevated levels of RDP ($P \leq .02$). The biggest incremental response for all of these fatty acids was between 80 and 120 g RDP/d, which indicates that a N deficiency at the lower supplemental levels limited rumen fermentation.

Conclusion

From the results of the present study it was clear that RDP supplementation can enhance rumen fermentation and MP synthesis on low-quality roughages and thereby address intake and digestibility limitations. Digestible OM intake increased in a quadratic fashion ($P = .01$) as the level of RDP was elevated. The estimated RDP requirement for Dohne Merino wethers, fed wheat straw, to maximise DOMI was 3.15 g/kg $\text{BW}^{.75}$ or 11.6% of DOM. This value corresponds well with the predicted RDP requirement of 11.1% of DOM for beef cows consuming low-quality forage (Köster *et al.*, 1996). These results are substantiated by a linear increase in total VFA concentration with increasing supplemental RDP levels. Despite the positive results observed in the present study, the practical problem of the high costs of protein supplementation still persists. Therefore, a visitation on substituting non-protein nitrogen (NPN) for natural protein in RDP supplements needs to be done.

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CHAPTER 3

Effect of Increasing Urea proportions in Rumen Degradable Protein Supplements on the Utilisation of Wheat Straw by Dohne Merino Wethers

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Abstract

Twenty five ruminally and duodenally cannulated Dohne Merino wethers were used to assess the effects of replacing casein with urea on a rumen degradable protein (RDP) equivalent basis on intake, fermentation and digestion characteristics. Animals had *ad libitum* access to low-quality wheat straw (3.2% CP; 74.2% NDF) and water. The control treatment provided all of the RDP in the form of calcium caseinate (90% CP; 100% rumen degradable). The percentages of supplemental RDP from urea in the other treatments were 25, 50, 75 and 100%. The 100% urea treatment was balanced with cornstarch to contain 40% CP and all other treatments received the same amount of starch (150 g/d). Forage organic matter (OM), forage N and total N intakes displayed a weak trend for a linear decrease with increasing urea levels ($P = .16$). Ruminal digestibilities of OM and NDF were not affected ($P \geq .18$) by urea level. Total tract OM digestibility decreased (linear; $P = .10$) and NDF digestibility tended to decrease in a linear manner ($P = .11$) with increasing urea proportions. As a result, digestible organic matter intake (DOMI) declined (linear; $P < .01$) with increasing urea levels. Effects of increasing urea levels on duodenal N flow, microbial efficiency and fluid dilution rate were minimal. Ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration tended to decrease quadratically ($P = .14$) with increasing urea levels, while total volatile fatty acids (VFA's) and rumen pH decreased in a linear manner ($P \leq .08$). Branched-chain volatile fatty acids (BCVFA's) and valerate decreased linearly ($P \leq .05$) with increasing urea levels, while other VFA's and acetate:propionate ratio were not affected by treatment ($P \geq .16$). It appears as if DOMI, as well as all ruminal and total tract OM and NDF digestibility criteria, reached

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their highest values at 25% of the RDP from urea. Therefore, it is concluded that replacing 25% of casein N with urea N in RDP supplements, will not impair the fermentation and digestive processes of sheep fed low-quality roughages.

Key Words: Rumen degradable protein, Urea, Intake, Wheat straw, Sheep

Introduction

Proteolytic and deaminative enzymes of rumen micro-organisms degrade dietary protein to volatile fatty acids (VFA's), peptides, amino acids and ammonia (Kang-Meznarich & Broderick, 1981). Ammonia (NH₃) is the primary N source for the growth of rumen micro-organisms (Virtanen, 1966; Nolan, 1975; Aharoni *et al.*, 1991) and is essential for the growth of several species of rumen bacteria (Allison, 1970; Bryant, 1973). Bryant & Robinson (1962) stated that 82% of rumen bacteria can grow with NH₃ as the sole source of N, 25% would not grow unless NH₃ was present, and 56% could utilise either NH₃ or amino acids.

When ruminants subsist on low-quality roughages, biochemical conditions in the rumen supposedly are not conducive to the use of high levels of urea. Cellulose may be fermented too slowly to furnish an adequate energy supply to keep pace with the rapid release of NH₃ from urea hydrolysis. This may be a reason why ruminants usually perform better when dietary N is present in the form of protein (Stock *et al.*, 1986; Rooke & Armstrong, 1989). In addition, protein provide pre-formed peptides and amino acids which can be directly incorporated into microbial protein (MP), with a subsequent lower energy cost and/or some of the dietary protein can escape to the abomasum. Nevertheless, it is generally accepted that rumen degradable protein (RDP) is beneficial to rumen micro-organisms and will enhance fibre digestion and efficiency of microbial growth compared to NH₃ alone (Rooke & Armstrong, 1989; Merry *et al.*, 1990; McAllan, 1991). In addition, Freeman *et al.* (1992) suggested that with respect to fibre digestion, the source of supplemental CP could be more important than the percentage of CP in supplements or feeding level.

Russell *et al.* (1992) reported that bacteria which degrade starch and sugars require peptides and amino acids for optimal growth, while cellulolytic bacteria use NH₃ as a chief N source. Therefore, on low-quality N deficient roughages, nonprotein nitrogen (NPN) should be able

to replace at least a portion of the RDP without sacrificing animal performance. Such substitution should be on a RDP equivalent basis, while a readily fermentable carbohydrate source should be provided as well, in order to synchronise energy and N release. The objective of this study was to determine the effect of increasing proportions of supplemental N from urea in isonitrogenous supplements on forage intake, digestion and rumen fermentation characteristics.

Material and Methods

Twenty-five rumen and duodenally cannulated Dohne Merino wethers were used in a total randomised block design. Animals were housed individually in 1 x 2 m metabolism cages and had *ad libitum* access to wheat straw (3.2% CP; 74.2% NDF) and water. The wheat straw was chopped into 5 cm pieces with a hammermill and fed at 140% of the average previous five day intake of each individual animal, just after intraruminal administration of the supplements. There were 5 treatments, with the control treatment providing the required amount of RDP (3.15 g/kg W^{0.75}) to maximise digestible organic matter intake (DOMI), in the form of calcium caseinate (90% CP; 100% rumen degradable). The other four treatments respectively provided 25, 50, 75 and 100% of the supplemental RDP from urea. Supplements were dissolved in 600 mL water and administered at 07h00 and 19h00, just before feeding wheat straw. To prevent possible trace- and macro mineral deficiencies, a salt-mineral mixture (26% NaCl, 16% Ca, 8% P, 0.004% I₂, 0.06% Cu, 0.001% Co, 0.25% Mn and 0.22% Zn), formulated in accordance with the mineral content of wheat straw, was administered intraruminally at 19.05 g/sheep/d with the morning supplements.

Table 1. Composition of supplements on a DM basis

Ingredient (g DM/d)	Treatment				
	0% Urea	25% Urea	50% Urea	75% Urea	100% Urea
Casein	77.5	58.1	38.8	19.4	0
Urea	0	6.1	12.1	18.2	24.3
Maize Starch	150	150	150	150	150

Results on urea toxicity are somewhat variable, since Helmer & Bartley (1971) reported incidents where toxicity were only elicited when rumen ammonia nitrogen (NH₃-N)

concentrations exceeded 1 760 mg/L. In contrast, the same authors reported that the capacity of the liver to convert NH_3 to urea is exceeded when rumen fluid contains more than 840 mg $\text{NH}_3\text{-N/L}$. The rumen $\text{NH}_3\text{-N}$ concentrations in the current study were much lower than the above mentioned ones, but Helmer & Bartley (1971) also reported the incidence of NH_3 toxicity in sheep to occur at urea levels of .28 - .44 g/kg BW. In the present study the 100% urea treatment provided .61 g urea/kg BW, and because of the low rate of digestion and slow fermentation of wheat straw (Doyle & Panday, 1990), the incidence of NH_3 toxicity was a possibility with the administration of higher urea supplements.

In order to supply a fast releasing energy source with the fast release of N from urea, maize starch was included to balance treatment 5, the supplement with the highest amount of urea, to 40% CP content. The other treatments were formulated to have the same amount (150 g/d) of starch as the 100% urea treatment, in order to prevent possible differences in fermentation and digestion of the wheat straw as a result of different starch inclusion levels (Sultan & Loerch, 1992; Olson *et al.*, 1999). For the first three days of the trial animals were observed carefully for any symptoms of NH_3 toxicity, until it was certain that they have adapted to the high urea levels. Secondly, starch was included in the supplements, since the final practical application of this type of supplementation information will entail the provision of supplemental N with starch or other readily digestible carbohydrates. Competition for nutrients between cellulolytic and amylolytic bacteria in the rumen has been noted as the predominant cause for the inhibition of cellulose digestion in the presence of starch (El-Shazly *et al.*, 1961). However, the same authors reported that these detrimental effects were absent when sufficient N was supplied.

Sampling

The sheep were allowed to adapt to the diet and their specific supplements for the first 14 d of each period. During the following 4 d, voluntary feed intake was measured and representative wheat straw samples were taken at each feeding time. Orts were removed before the morning feeding and kept as individual samples for each animal. From d 16 through 18, duodenal and faecal samples were taken at 6 h intervals, starting at 24h00. Sampling time was advanced 2 h each day to obtain samples on every even hour of a 24 h period. On d 19 the rumen of each sheep was completely emptied at 06h00, just before

feeding and infusion of supplements, and again at 15h00, to obtain a representative sample of rumen contents. The content was weighed, mixed and a 1 kg sample was frozen immediately.

At 06h00 on d 20 of the trial, 15 mL of Cr-EDTA, (360 mg Cr/15 mL; Uden *et al.*, 1980) was infused into various ruminal sites to determine the fluid dilution rate. Sixty mL of ruminal fluid was extracted at 0, 3, 6, 9, 12 and 24 h and the pH of these samples were measured at the time of extraction from the rumen. Ruminal fluid samples obtained at 0, 3, 6, 9 and 12 h were preserved to determine VFA and NH₃-N concentrations. Nine mL rumen fluid plus 1 mL of a 10% (m/v) NaOH solution was used to determine the VFA concentration (Cottyn & Bouche, 1968) and 8 mL of a .1 N HCl solution was added to 2 mL rumen fluid to determine rumen NH₃-N concentrations (Broderick & Kang, 1980). All samples were frozen immediately to be analysed later.

A specially developed device was used for the ruminal fluid extractions. This apparatus consisted of the casing of a 5 mL syringe, inserted into an *in situ* degradation bag with 50 μ diameter pores. The syringe had 25 pores with a diameter of 3 mm and the open end was blocked with a rubber stopper. A 20 cm long rubber tube was connected to the nozzle of the syringe and passed through the cork of the rumen fistula. The tube was connected externally to a small tap which could be attached to a 60 mL syringe to extract ruminal fluid. Merits for using this apparatus are that the rumen fistulas are not opened for sampling, but only the tap, and thus rumen conditions are kept anaerobic and constant. In addition, ruminal fluid obtained in this manner is clean and contains no large feed particles.

Laboratory Analyses

Faecal samples were dried in a normal drying oven for 96 h at 50°C. Feed, orts and faecal samples were ground with a Scientec Hammer Mill (Peter Rassloff, Instruments & Services (PTY) Ltd.) to pass a 1 mm screen. Faecal and orts samples were pooled for each wether within each period, while the 5 dietary samples for each period were pooled to form 1 representative feed sample. These samples were analysed for N, NDF and acid insoluble ash (AIA). Acid insoluble ash (Cottyn & Bouche, 1968; .2 N HCl procedure) was used as a natural marker to calculate digestibility coefficients. All N and NDF analyses were done

according to the methods of the AOAC (1984) and N was measured with a Leco Auto Analyser (Model FP 428). For determination of DM, feed, orts and faecal samples were dried at 100°C in a convection oven. Samples were ashed for 5 h at 500°C to determine organic matter (OM) concentrations (AOAC, 1984).

Duodenal samples were lyophilised and then pooled for each wether within each period. These pooled samples were finely ground with a Scientific Apparatus sample mill (Arthur H. Thomas Co., Philadelphia, PA) and analysed for N, NDF, AIA and purine concentration (Zinn & Owens, 1986). For determination of NH₃-N concentrations in the duodenum, samples were reconstituted to 3% DM in a .1 N HCl solution, mixed and centrifuged at 20 000 x g for 20 min (Hannah *et al.*, 1991). The supernatant was then poured off and analysed for NH₃-N according to the phenol hypochlorite procedure described by Broderick & Kang (1980).

Ruminal fluid samples for determination of VFA's and NH₃-N were thawed at room temperature. Ammonia N samples were analysed according to the aforementioned procedure described by Broderick & Kang (1980) and VFA concentrations were measured by gas chromatography. Chromium concentration in ruminal fluid samples were diluted (1:2 dilution) and the Cr concentration measured with a radial emission, inductively coupled plasma spectrometer (Liberty Series II). Fluid dilution rate was determined by regressing the natural logarithm of Cr concentration on sampling time (Warner & Stacy, 1968).

Rumen bacteria were isolated by adding a saline solution (9 g NaCl/L; 500 mL/kg rumen content) to the rumen contents and blending the mixture for approximately 1 min. The samples were then strained through 2 layers of cheesecloth and centrifuged at 1000 x g for 10 min to remove feed particles and protozoa. The supernatant was poured off and centrifuged at 20 000 x g for 20 min to isolate the bacteria. Once again the supernatant was poured off, the pellets were washed with saline solution (9 g NaCl/L), combined and re-centrifuged at 20 000 x g for 20 min. Isolated bacteria were frozen, lyophilised and analysed for purine concentration.

Statistical Analysis

Intake, digestibility and flow data were analysed as a randomised block, using the GLM procedure of SAS (1994), with effects for animal, block and treatment. Treatment sums of squares were partitioned into linear, quadratic and cubic effects of RDP level with orthogonal polynomials.

Rumen pH, VFA's and NH₃-N were analysed as a randomised block split-plot design. Whole-plot sources of variation were animal, block and treatment, and the subplot sources were time and treatment x time interactions. Treatment sums of squares were partitioned into linear, quadratic and cubic contrasts.

Results and Discussion

Intake and Digestibility

Forage OM intake, forage N intake and total N intake (Table 2) showed a slight tendency of a linear decrease ($P = .16$) with increasing urea levels. Total OM intake and DOMI decreased significantly (linear; $P \leq .02$) with increasing urea proportions. This depression may result from a reduced availability of amino acids and peptides, or other microbial growth factors, since Bryant (1973) and Hoover (1986) reported that cellulolytic microbes require valerate and/or branched-chain volatile fatty acids (BCVFA's) for growth. In the present study, molar percentages of the BCVFA's and valerate decreased linearly with increasing urea levels ($P \leq .05$). It is concluded that increasing NPN proportions in RDP supplements fed to sheep consuming wheat straw, will tend to inhibit voluntary feed intake. Results of Kempton & Leng (1979) verified these findings, by stating that NPN alone cannot support maximum feed intake. The response in DOMI is in contrast with the results of Köster *et al.* (1997), who reported a decrease only at the 100% urea supplement to beef cattle consuming low-quality forage.

Apparent ruminal OM, true ruminal OM and ruminal NDF digestibilities were not significantly affected by treatment ($P \geq .18$). Egan & Doyle (1985) verified this absence of a response in ruminal digestibility in the present study, by stating that intraruminal urea

supplementation did not result in clear evidence of a change in the digestibility of OM or in the rate of digestion of cell wall constituents. In contrast to ruminal digestibility, total tract OM and NDF digestibilities tended to decrease as the proportion of N from urea was increased ($P \leq .10$). This leads to the assumption that increasing levels of NPN in isonitrogenous RDP supplements fed to sheep consuming wheat straw, could have a more pronounced effect on total tract digestibility than on rumen fermentation. Kropp *et al.* (1977) and Kempton & Leng (1979) confirmed this, since these authors reported no improvement in ruminal OM digestibility in response to increasing urea levels fed to steers and lambs. In contrast, Köster *et al.* (1997), who replaced casein with urea on an isonitrogenous basis in RDP supplements fed to steers, reported fairly constant responses for ruminal as well as total tract OM and NDF digestibility parameters up to 75% N from urea. However, they observed a negative response with the highest urea inclusion rate.

Urea is hydrolysed about 4 times faster than NH_3 is absorbed (Bloomfield *et al.*, 1960). Therefore a possible explanation why rumen digestibilities were not significantly affected by higher urea levels, is that the maize starch provided a readily fermentable energy source with the quick release of NH_3 from urea. This would result in an enhanced efficiency of N utilisation at higher urea levels. In addition, Chalmers *et al.* (1976) noted that a rumen $\text{pH} < 7$ is not conducive to passive absorption of NH_3 from the rumen into the bloodstream. Since the maximum rumen pH in the present study was $6.76 (\pm .05)$, it can be concluded that most of the NH_3 in the rumen would have been available for utilisation by the rumen microbes. The fact that $\text{NH}_3\text{-N}$ flow to the duodenum did not increase with higher urea levels ($P \geq .2$), supports this argument.

Despite the observed linear decrease in DOMI, associated with increasing urea inclusion levels, the corresponding value for the 25% urea N treatment appears to be higher than any of the other treatments. This response is explained by the fact that forage OM intake as well as ruminal and total tract digestibilities of OM and NDF appear to have reached a numerical maximum at 25% urea inclusion. It seems therefore, that rumen fermentation as well as total tract digestive processes are enhanced by providing 25% of the RDP requirement from NPN. This is in contrast to the results of Köster *et al.* (1997), who indicated that 50% of true protein can be replaced by NPN in order to maximise DOMI in beef cattle fed low-quality roughages.

Keeping the high cost of natural resources in mind, Köster (2000) suggested that depending on the magnitude in the decrease of forage utilisation with increasing urea proportions, the

Table 2. Effect of increasing proportions of supplemental N from urea on intake, digestibility and duodenal flow in Dohne Merino wethers fed wheat straw

Parameter	% Supplemental N from Urea					SEM ¹	Contrasts ²		
	0	25	50	75	100		L	Q	C
OM Intake	g/kg BW ^{.75}								
Forage	38.79	41.90	39.15	36.78	30.94	4.53	.16	.30	.81
Supplement	15.09	13.28	12.59	11.13	10.07				
Total	53.88	55.18	51.74	47.91	41.01	4.05	.02	.26	.83
Digestible OM Intake	30.95	34.41	24.60	23.39	20.15	2.14	<.01	.52	.11
N Intake	g/kg BW ^{.75}								
Forage	.25	.27	.25	.24	.20	.03	.16	.30	.81
Total	1.00	.99	1.00	.97	.95	.02	.16	.45	.75
Ruminal Digestibility	%								
Apparent OM	45.82	59.43	44.66	42.51	46.92	7.16	.62	.92	.19
True OM	49.47	59.71	49.41	47.55	51.18	5.44	.64	.83	.18
NDF	55.91	62.87	50.82	52.03	54.92	4.65	.42	.74	.22
Apparent N	12.48	23.62	18.21	17.72	36.85	13.07	.27	.60	.49
Duodenal Flow	g/d								
Total N	9.59	10.14	9.80	9.19	7.59	1.34	.27	.38	.95
Microbial N	5.93	6.03	7.53	6.52	5.43	1.02	.86	.20	.70
Ammonia N	.83	.91	.90	1.14	1.06	.17	.20	.96	.60
NMNAN ³	2.84	3.19	1.38	1.53	1.10	.37	<.01	.66	.29
Fluid Dilution Rate	%								
	6.75	6.35	7.59	7.09	6.62	.63	.78	.45	.36
Microbial Efficiency	g N synthesised/kg OM truly digested								
	20.09	13.98	24.57	23.57	21.43	3.33	.29	.72	.12
Total tract digestibility	%								
OM	57.44	62.36	47.53	48.82	49.12	5.08	.10	.63	.36
NDF	61.93	65.80	52.08	53.02	54.08	4.97	.09	.61	.33
N	62.48	63.20	57.00	62.65	68.48	4.90	.44	.22	.74

¹Standard error of the mean (n=5).

²L = linear, Q = quadratic, C = cubic.

³Nonmicrobial-nonammonia N.

economically feasible level of urea inclusion will depend on the cost of the different raw materials used in the supplement. Maeng *et al.* (1976) found that a combination of NPN and natural protein enhanced MP production and that the ratio of NPN to amino acid-N is critical in maximising microbial growth. In *in vitro* studies the optimum ratio of NPN:amino acid-N was 75:25, while both 100% urea and 100% amino acid failed to yield maximum microbial growth. When amino acids replaced 25% of urea-N *in vitro*, the specific growth rate of

rumen bacteria was doubled (Maeng & Baldwin, 1976). Conversely, in diets containing 17.5 - 18% CP, urea N was used less efficiently for microbial growth than natural protein, presumably resulting from a more rapid initial NH₃ release (Stern *et al.*, 1978). Reasons why sheep appear to have a substantially higher supplemental true protein requirement than beef cattle are not quite clear and need further investigation.

Rumen Fermentation characteristics

Coombe & Tribe (1962) suggested that urea has a more pronounced effect on retention time than on digestibility. They argued that for low-quality forages such as wheat straw, which normally moves slowly through the gut, a high proportion of the digestible material is broken down in a relatively short time, compared to the time that the material spends in the rumen. Thus the advantages gained by increasing the rate of passage of such food through the gut are not lost by a reduction in digestibility. To the contrary, the highest level of NPN supplementation in the present study (24.3 g urea/d) did not affect fluid dilution rate (cubic; $P = .36$). The lack of change in fluid dilution rate in the present study, is in agreement with the minor effects of treatment on digestion and feed intake.

According to Coombe *et al.* (1960), the predominant factors influencing passage rate are feed intake, rumination and rumen motility. Time spent ruminating is reduced with a rumen pH in excess of 7.2 and NH₃-N concentrations of more than 885 mg/L, while rumen motility is inhibited by a rumen pH of more than 7.15 and NH₃-N concentrations in excess of 1300 mg/L (Coombe *et al.*, 1960). In the present study, the maximum recorded rumen pH was 6.76 ($\pm .05$) with a corresponding NH₃-N level of 257.16 mg/L (± 19.13). Therefore it appears that these levels were not high enough to have any significant negative effect on rumination or rumen motility.

Although nonmicrobial-nonammonia N (NMNAN) decreased with increasing urea levels ($P < .01$) and efficiency of microbial protein (MP) synthesis showed variable results (cubic; $P = .12$), total N, microbial nitrogen (MN) and NH₃-N flow to the duodenum were not affected by different NPN levels in isonitrogenous RDP supplements ($P \geq .2$). These nutrient flow data correspond well with the lack of response in the rumen digestibility criteria. In addition, the general lack of effect on microbial fermentation in the present study

agrees with that of Köster *et al.* (1997), that reported only minor effects on microbial N, NH₃-N and efficiency of MP synthesis when replacing natural protein with urea. Similarly, Oh *et al.* (1969) maintained that substituting casein for urea did not significantly alter MP concentration

Microbial efficiency is expressed as g MN synthesised/kg OM truly digested and therefore the increased ruminal and total tract digestibilities, as well as DOMI at the 25% urea N level observed in this study, may explain the reduced microbial efficiency for that treatment. Except for the 25% urea N treatment, microbial efficiency was fairly similar among treatments and corresponded well with the reported 21.3 g MN synthesised/kg OM digested when sheep were fed a virtually protein free diet, supplemented with 16g urea (Hume *et al.*, 1970).

Table 3. Effect of increasing proportions of supplemental N from urea on pH, NH₃-N and VFA concentrations in the rumen of Dohne Merino wethers fed wheat straw

Parameter	% Supplemental N from Urea					SEM ¹	Contrasts ²		
	0	25	50	75	100		L	Q	C
PH	6.52	6.74	6.65	6.73	6.76	.05	.08	.54	.40
NH ₃ -N (mg/L)	168.81	130.46	160.36	180.22	257.16	19.13	.28	.14	.86
Total Volatile Fatty Acids (mM)	63.62	54.75	54.54	52.78	50.32	2.12	.03	.46	.47
	Mol/100 mol								
Acetate	65.28	67.27	64.56	69.01	63.98	1.47	.92	.40	.43
Propionate	22.19	21.62	23.02	20.58	25.83	.99	.32	.16	.18
Butyrate	7.20	6.73	8.52	7.39	7.10	.37	.89	.35	.64
Isobutyrate	1.58	1.37	1.19	0.78	1.05	.14	<.01	.17	.16
Valerate	1.55	1.27	1.33	1.12	1.00	.08	.05	.98	.70
Isovalerate	1.86	1.43	1.03	.70	.60	.07	<.01	.83	.37
Hexanoic Acid	.35	.32	.34	.41	.44	.04	.34	.70	.66
Acetate:Propionate	3.06	3.23	3.05	3.67	2.69	.17	.76	.17	.17

¹Standard error of Mean

²L=linear; Q=quadratic; C=cubic

According to the results from Table 3, increasing urea levels in RDP equivalent supplements showed a weak trend to increase rumen NH₃-N level in a quadratic fashion (P = .14). Although treatments were isonitrogenous, urea is fermented at a faster rate than casein, with a subsequent quicker release of NH₃ with higher urea levels. Ammonia may thus be produced at a faster rate than it can be utilised for MP synthesis, resulting in somewhat higher NH₃-N concentrations, as observed in this study. According to Satter & Slyter (1974) and

Slyter *et al.* (1979) the rumen $\text{NH}_3\text{-N}$ requirement for maximum MP production is 20 - 50 mg/L. Therefore, the $\text{NH}_3\text{-N}$ concentrations in the present study appeared to be adequate for maximum MP production for all treatments. Although the highest $\text{NH}_3\text{-N}$ concentrations in this study were observed with the highest urea levels, the lack of treatments effects on rumen digestibility parameters indicate that NH_3 was probably not limiting microbial fermentation. Erdman *et al.* (1986) reported that the minimum $\text{NH}_3\text{-N}$ required for maximum DM disappearance depends upon the fermentability of the feed. They concluded that low-quality feeds generally require lower $\text{NH}_3\text{-N}$ concentrations for maximum digestion than feeds of higher fermentability.

Total VFA concentration decreased linearly as urea level increased ($P = .03$), which verifies the general accepted view that natural protein is more beneficial to rumen fermentation than NPN. Peptides and amino acids from natural protein are directly incorporated into MP (Nolan *et al.*, 1976), with a subsequent lower energy cost compared to NH_3 , in which case the amino acids have to be synthesised. In addition, deamination of certain amino acids provide BCVFA's that serve as microbial growth factors to cellulolytic micro-organisms (Hoover, 1986). The response in total VFA concentration is in agreement with rumen pH values, which increased linearly ($P \leq .08$) from a minimum of 6.52 for the 0% urea treatment, to a maximum of 6.76 for the 100% urea treatment. These values are close to a pH of 6.5, which is regarded as the optimum for cellulose and OM fermentation (Hoover *et al.*, 1984). In contrast, Kempton & Leng (1979) reported that the rate of VFA production (mol/MJ ME intake) on a low-quality roughage diet, was numerically higher with urea supplementation than with either urea plus casein, or urea plus formaldehyde-treated (HCHO)-casein. In addition, Oh *et al.* (1969) and Köster *et al.* (1997) reported that replacing urea with an isonitrogenous amount of casein did not significantly increase VFA production.

Molar proportions of acetate, propionate, butyrate and hexanoic acid, as well as the acetate:propionate ratio were not affected by different urea levels in RDP equivalent supplements ($P \geq .16$). Molar percentages of valerate, isovalerate and isobutyrate decreased in a linear fashion ($P \leq .05$) as the percentage of urea increased. This corresponds with the reduced DOMI and total tract OM and NDF digestibilities ($P \leq .10$) as urea levels were elevated. In contrast Kempton & Leng (1979) reported that supplements containing 25 g urea

plus 150 g casein/kg forage, significantly increased molar proportions of branched chain and higher fatty acids in lambs compared to urea and urea plus HCHO-casein.

Conclusion

Substituting casein for urea on an isonitrogenous basis in RDP supplements generally decreased DOMI and total tract digestibility of OM and NDF, while ruminal digestibility parameters were not altered. However, it appears as though the highest values for ruminal and total tract digestibilities of OM and NDF, as well as for DOMI, were obtained by replacing 25% of the supplemental casein N with urea N. It is concluded that 25% of the true protein in RDP supplements provided to sheep consuming low-quality forages, can be substituted by urea without any detrimental effect on intake and digestibility. Higher inclusion levels may, however, impair forage utilisation and thus animal performance. To ensure maximum intake, supplement palatability must be considered during final evaluation of the potential for urea inclusion in supplements for sheep fed low-quality roughages.

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CHAPTER 4

General Conclusion

Despite the readily availability of low-quality, high roughage forages, the utilisation of these forages by ruminants are limited, due to a low digestibility and a N deficiency. In addition, low-quality forages are unpalatable, resulting in low voluntary feed intakes, rendering it unsuitable to sustain productive animal production. Since N is generally considered as the first limiting nutrient to ruminants grazing low-quality forage, the most efficient way of improving the utilisation of low-quality roughages is N supplementation.

Various research studies have indicated that true protein supplements are more beneficial to rumen fermentation than nonprotein nitrogen (NPN). Therefore rumen degradable protein (RDP) supplements (calcium caseinate; 90% CP; 100% rumen degradable) were used to determine the supplemental protein level where digestible organic matter intake (DOMI) was maximised. Maximum DOMI was achieved at an estimated supplemental RDP requirement of 3.15 g/kg BW^{0.75} or 11.6% of DOM.

Based on the high cost of natural protein supplementation and the fact that rumen microbes can grow with ammonia (NH₃) as the sole N source, substituting urea for casein at different levels, ranging from 0 - 100%, on an isonitrogenous basis, was also investigated. In corroboration with earlier research, it appeared as if a combination of NPN and true protein furnished the highest DOMI. Replacing 25% of the casein N with urea N tended to increase DOMI and ruminal and total tract digestibility characteristics. It was concluded that supplementing 3.15 g RDP/kg BW^{0.75} or 11.6% of DOM, constituted from 75% true protein and 25% urea, will be the most economically feasible supplementation strategy for maximising DOMI and maintaining effective utilisation of low-quality forages by sheep.

The optimum supplemental N requirement for maximum DOMI is now determined. However, animal performance and low-quality roughage utilisation may be further enhanced with effective energy supplementation strategies. The field of supplemental energy level as well as an evaluation of different energy sources in conjunction with the provision of the optimum supplemental N level holds considerable research opportunities. For maximum

exploitation of low-quality forages in practical animal production, further research in the field of energy supplementation to ruminants grazing low-quality forages is recommended.