

USE OF PRO-SIL-TREATED CORN
SILAGE AND FABABEAN SILAGE IN
RATIONS FOR LACTATING
DAIRY COWS

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by
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DEDICATED TO MY PARENTS

ABSTRACT

USE OF PRO-SIL-TREATED CORN SILAGE AND FABABEAN SILAGE
IN RATIONS FOR LACTATING DAIRY COWS

Felix Budara Bareeba

In two experiments, corn silages were treated at harvest with urea (0.5% wet basis) or Pro-Sil (1.3 to 2.2% wet basis). Recoveries of added nitrogen (N) from silage ranged from 95 to 100%. Water insoluble N and lactic acid contents were higher in the NPN-treated corn silages compared to untreated corn silage.

Three wethers were fed grass-legume (GL) silage (38% DM), urea-treated corn silage (31% DM) and Pro-Sil-treated (2.2%) corn silage (32% DM) in a digestibility and N balance trial. No significant ($P > .05$) differences were observed for silage dry matter (DM) consumption and N utilization among treatments.

Eight lactating Holstein cows were fed four diets in a change-over design. Diets were GL silage + medium grain (MG); urea-treated corn (UC) silage + MG; Pro-Sil-treated (2.2%) corn (PC) silage + MG and PC silage + low grain (LG). Cows received GL, UC and PC silages ad lib plus MG in a 60:40 (DM) ratio and PC silage plus LG in a 70:30 (DM) ratio. No significant ($P > .05$) differences were noted among treatments for silage DM consumption, milk yield and milk composition. The apparent digestibilities of DM and energy were lower ($P < .05$) for the GL silage + MG diets compared to the other

diets. Cows fed the GL silage + MG diet had higher ($P < .05$) blood urea-N levels than those fed the PC silage-containing diets.

Four wethers were fed untreated corn (C) silage (38% DM), UC silage (32% DM), PC (1.3%) silage (42% DM) and PC (1.7%) silage (32% DM) in a digestibility and N balance trial. Silage DM consumption was lower ($P < .05$) for the UC silage compared to the other silages. The apparent digestibility of crude protein (CP) was lower ($P < .05$) for the C silage compared to the other silages as expected with differences in protein content of the silages. The apparent digestibility of acid-detergent fibre (ADF) was higher ($P < .05$) for the PC (1.7%) silage than for the other silages. Although sheep fed the C silage consumed less ($P < .05$) N, N retention (% of N intake) was not different ($P > .05$) among treatments. Rumen ammonia ($P < .01$) and blood urea-N ($P < .05$) were lower for sheep fed the C silage than for those fed the other silages.

Eight lactating Holstein cows were fed two corn silages (C and PC, 1.3%) and four grain mixtures containing either 6.4% SBM (#1), 12.5% SBM + 1.3% urea (#2), 40% fababeans (FB) + 0.6 encapsulated methionine (#3) or 42% FB (#4) in a 45:55 (DM) ratio as a complete feed in a change-over design. Diets were Pro-Sil (PC silage + grain #1); Urea (C silage + grain #2); Fababean + methionine (C silage + grain #3) and Fababeans (C silage + grain #4). Silage DM consumption was lower ($P < .05$) for cows fed the Pro-Sil diet than for those fed

the fababean-containing diets. Milk and FCM yields, protein and solids-not-fat contents were not different ($P > .05$) among treatments. Milk fat test was lower ($P < .05$) for cows fed the Pro-Sil diet than for those fed the Fababean diet. Substituting SBM with fababeans decreased ($P < .05$) ration DM digestibility. The apparent digestibility of energy was lower ($P < .05$) for cows fed the Fababean + methionine diet than for cows fed the Urea diet. Methionine supplementation (15g Met/day) had little effect on feed consumption, milk yield, milk composition, plasma free methionine levels and Met/Val ratios.

In two other experiments, the conservation of whole plant fababean as untreated direct-cut (FB), untreated wilted (WFB) and formaldehyde-treated (1.2% DM) (FFB) silage was studied. Wilting and formaldehyde treatment did not restrict silage fermentation. All silages had high acid-detergent insoluble N (ADIN) in the dry matter indicative of heat damage.

Twelve lactating Holstein cows were fed four diets, GL silage (35% DM) + high grain (HG), FB silage (33% DM) + HG, WFB silage (37% DM) + HG and WFB silage + medium grain (MG) in a Lucas design. Consumption of the FB silage was higher ($P < .05$) than that of the GL silage, and reducing the level of grain feeding from 56 to 43% of the diet resulted in an increase ($P < .01$) in the WFB silage consumption. Milk and FCM yields and milk composition were not different ($P > .05$) among treatments.

Eight lactating Holstein cows were fed either FB (33% DM) or FFB (31% DM) silage plus a dairy concentrate in a 45:55 (DM) ratio as a complete feed in a change-over design. The cows were supplemented with or without 13g/day methionine in the form of encapsulated methionine. Silage DM and total DM consumption, milk yield and milk composition were not different ($P > .05$) among treatments. Formaldehyde treatment decreased ($P < .05$) the apparent digestibilities of ADF and energy. Methionine supplementation had little effect on feed consumption, milk yield, milk composition, plasma free methionine levels and Met/Val ratios.

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LIST OF ABBREVIATIONS

α	Alpha
<u>ad lib.</u>	<u>Ad Libitum</u>
ADIN	Acid-detergent insoluble nitrogen
β	Beta
BUN	Blood plasma urea-nitrogen
DAP	Diaminopimelic acid
DM	Dry matter
DMI	Dry matter intake
ϵ	Epsilon
FB	Fababean silage
FCM	Fat-corrected milk
FFB	Formaldehyde-treated fababean silage
γ	Gamma
g	gram
GL	Grass-legume silage
Kg	Kilogram
l	liter
μ M	Micromoles
M	Molar
m	meter
Met	Methionine
MHA (M-analog)	Methionine hydroxy analog
mg	Milligram
ml	Milliliter
mM	Millimoles

N	Nitrogen
NPN	Non-protein nitrogen
NH ₃ -N	Ammonia-nitrogen
WSN	Water-soluble nitrogen
NGR	Non-glucogenic ratio
OM	Organic matter
%	Percent
PC	Pro-Sil-treated corn silage
RAN	Rumen ammonia-nitrogen
NaCl	Sodium chloride
SNF	Solids-not-fat
SBM	Soybean meal
C	Untreated corn silage
UC	Urea-treated corn silage
Val	Valine
VFA	Volatile fatty acids
W ^{3/4}	Metabolic body weight
WFB	Wilted fababean silage
WSC	Water soluble carbohydrates

INTRODUCTION

The high levels of fermentable carbohydrates in corn plant material will normally ensure that adequate quantities of lactic acid are produced by fermentation to give good preservation when the crop is ensiled. However, in order to achieve high levels of production from lactating dairy cows fed corn silage, it is generally necessary to provide an additional nitrogen supplement. Urea has traditionally been used for this purpose; however, other sources such as gaseous ammonia or an ammonia-molasses-mineral solution (Pro-Sil)¹ have received considerable attention recently.

American workers have shown that ammonia or Pro-Sil has a better influence on the fermentation process than urea resulting in a higher production of lactic acid, increased stability and better feeding results. The aim of the first part of this study was to compare the effect of Pro-Sil with that of urea on the fermentation process and nutritive value of a typical forage corn crop grown under Canadian conditions.

Fababean (Vicia faba L.) has been recently introduced into Canada from Europe as a protein extender in livestock feeding. Limited information is available regarding the utilization of whole plant fababean as a feed for ruminants. The yield potentials of the crop suggest that the whole plant

¹ Trade name Pro-Sil, a product of Ruminant Nitrogen Products Company, Okemos, MI 48864. Contains 13.6% N (as ammonia), molasses, NaCl, CaCl, plus Ca, P, S, Mg, Zn, Cu, Co, and I.

could be an economical feed when used either as a silage or a dehydrated product. The objective of the second part of this study was to determine the nutritive value of whole plant fababean silage compared with grass-legume silage for lactating dairy cows.

REVIEW OF LITERATURE

The Role of Silage Fermentation on
Nitrogen Utilization

Nitrogen Degradation during Ensiling: Upon ensiling and also during wilting (Brady, 1960; 1965) of a hay crop, plant protein (acid-precipitable or water-insoluble N) is degraded into water soluble low molecular weight compounds. Brady (1960) working with grass and leguminous fodder plants, showed that under conditions of ensilage and slow wilting, there is a marked increase in non-protein nitrogen (NPN). Both wilting and ensiling resulted in a rapid increase in free α - amino nitrogen concentration. The extent of protein degradation (proteolysis) is dependent upon the dry matter (DM) of the whole plant material at the time of ensiling (Hawkins et al., 1970; Brady, 1965).

Ammonia nitrogen ($\text{NH}_3\text{-N}$) content has been used as an index of proteolysis in grass silages. As silage DM decreased from 64.8 to 38.6%, $\text{NH}_3\text{-N}$ (% total N) increased from 5 to 15% (Gordon et al., 1965). For a direct cut alfalfa silage (20% DM), the $\text{NH}_3\text{-N}$ (% total N) level was 20.7% while a 53% DM wilted alfalfa haylage had an $\text{NH}_3\text{-N}$ level of 9.5%. For corn plant material after ensiling, early harvested material (20% DM) had a tungstic acid soluble N level (% total N) of 52% while late cut material (50% DM) had a tungstic acid soluble N level of about 38% (Johnson et al., 1967).

Since under field conditions, silage DM is a consequence of maturity, the role of DM per se (at same maturity of the

whole plant material) in proteolysis has been investigated. Hawkins et al., (1970) harvested direct cut alfalfa in early bloom stage. Some of the material (22% DM) was ensiled directly, while the rest was partially air dried to either 44% or 80% DM and then ensiled. The partially dried material resulted in silage with lower water soluble N, NPN and $\text{NH}_3\text{-N}$ and less total organic acids than the direct cut material. Similar results were obtained by Bergen et al., (1974) with chopped whole corn plant material. From these results, it can be surmised that moisture level per se is a major factor controlling proteolysis in ensiled whole plant material. Presumably, the reduced moisture prevents activity of the proteases found in the plant material.

Many workers have considered this extensive proteolysis during ensiling a detriment to the feeding value of silages. Hence, an extensive number of approaches such as acid (mineral acids or formic acid), formaldehyde and alkali treatments have been applied to chopped whole plant material at ensiling. Generally with direct cut hay crop silages, formic acid treatment reduces silo storage losses, proteolysis, total organic acid levels and $\text{NH}_3\text{-N}$ content (Waldo, 1977). A similar effect on corn silage has also been noted. When low DM (24 - 28%) chopped whole corn plant material was treated with formic acid, proteolysis and lactic acid production decreased (Huber et al., 1972). Further work showed that formic acid was superior to acetic and propionic acid in preserving low DM corn silage (Huber et al., 1972). While formic acid treatment of hay crop

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silage generally improved animal performance, such an effect has not been noted for corn silage (Huber et al., 1972). The feeding value of high DM corn was preserved by formic acid treatment (Huber et al., 1972). The prevention of spoilage by formic acid of a silage of low fermentation potential is the likely reason for such an effect. But it appears that when applied at rates of 2-4 l/ton it is effective through lowering the pH of the crop ensiled rather than specific antimicrobial effects of the formic acid (Wilson and Wilkins, 1973; Woolford, 1975).

Formaldehyde application to direct cut hay crop decreased fermentation and increased silage intake by animals (Wilkinson et al., 1975). Formaldehyde treatment of low protein material such as ryegrass or the whole corn plant material has, however, had a negative effect. Although in ensiled whole corn plant material, fermentation and proteolysis were markedly depressed by formaldehyde, the plant protein was overprotected and became unavailable for utilization in the rumen and possibly elsewhere in the digestive tract (Wilkinson et al., 1975).

The mechanism of proteolysis in freshly cut whole plant material is a two-fold process. The initial breakdown of plant protein into peptides and amino acids is a rapid process and is caused primarily by endogenous proteases (Bergen et al., 1974; Watson and Nash, 1960; McDonald and Whittenbury, 1973) but further (secondary) transformations of amino acids are due largely to microbial activity (Voss, 1966; McDonald and Whittenbury, 1973).

Various workers have suggested that chemical transformations during the ensiling process may be important in determining silage consumption (Thomas et al., 1961; Wilkins et al., 1971; Geasler, 1970). Hence the pattern of plant protein proteolysis and further transformations of amino acids have been studied to evaluate the role of these changes on silage intake and animal performance. Upon fractionation, water soluble N in ensiled material was found to be composed of mainly amino acid N and peptide N, volatile amine-N and unidentified-N (Hughes, 1970). Hughes (1970) showed that in grass silages, this unidentified N fraction was largely composed of non-volatile amines arising from decarboxylation of amino acids by bacteria. Brady (1960) reported an increase in an 'unaccounted NPN' fraction during ensilage of grass and leguminous fodder plants. However, a complete characterization of the whole unidentified-N fraction has not been achieved.

Hughes (1970) also showed that non-volatile amine production occurs during storage (2 - 18 months) of even well preserved grass silage; however, early changes in composition of the water soluble N fraction in hay crop silages were not studied (Hughes, 1970). Bergen et al., (1974) studied the rate of proteolysis and changes in the composition of water soluble N in ensiled corn plant material over a period of 20 days with laboratory silos. Water soluble N (% total N) increased from 13.2% (day 0) to 41.6% (day 20). Amino acid-N increased while unidentified-N decreased and NH_3 -N showed no

trend. Similar results were reported by Buchanan-Smith and Yao (1978) who, upon further fractionation, showed that the unidentified N fraction was composed largely of peptide-N and amide-N.

Nutritional Value of Nitrogen Compounds in Ensiled Feedstuffs:

Studies have been done on proteolysis and secondary (microbial) N transformations in silages especially as they relate to the keeping quality of the silage. Hence, for hay crop silages, a high $\text{NH}_3\text{-N}$ content implied extensive deamination and an unstable, poorly preserved silage. For hay crop silages, excessive solubilization (degradation) of plant protein increases rumen microbial degradation and ammonia production so that urinary excretion is increased and N retention is decreased (Goering and Waldo, 1974). The above reasoning is correct as long as hay crop silage is the only feed given to the animals. This loss of ammonia could be offset however by increasing the digestible energy content of the ration. The N content of hay crop silage is in excess of its available digestible energy and hence ruminal utilization of soluble N is not very efficient. Under such circumstances, less proteolysis and enhanced ruminal bypass of forage protein would be advantageous.

Excessive insolubility of hay crop silage protein, caused by either heat damage (acid-detergent insoluble N formation) or overprotection by such treatments as formaldehyde or paraformaldehyde will depress performance since nitrogen availability to the animal is markedly reduced (Yu et al., 1977;

Wilkins et al., 1974a).

For corn silage, the above considerations must be modified. Corn silage is a low protein feed with a high digestible energy (TDN) content. Further, $\text{NH}_3\text{-N}$ usually comprises only about 10% of the water soluble N fraction of corn silage (Bergen et al., 1974). In corn silage the problem therefore is N bioavailability rather than inefficient utilization due to extensive $\text{NH}_3\text{-N}$ losses from the rumen.

Voluntary DM intake of ensiled plant material is less than that of fresh, frozen or dried companion forages (Gordon et al., 1961; Harris and Raymond, 1963; Dinius et al., 1968). Thomas et al., (1961) suggested that the DM content of the forage when ensiled and the resulting fermentation process are important factors determining the rate of consumption of silage. Two major compositional changes occur in the whole corn plant material during ensiling; namely the fermentation of water soluble carbohydrates into organic acids and the degradation of plant protein to nonprotein, water soluble N compounds (Johnson et al., 1967; Demarquilly and Andrieu, 1973; Bergen et al., 1974).

It appears that high acid content contributes to the low levels of consumption and consequently to low levels of production by animals fed on silage. McLeod et al., (1970) found that silage DMI was negatively correlated to silage pH, total organic acids and lactic acid. Addition of lactic acid to change the pH from 5.4 to 3.8 decreased silage DMI by 22%. In corn silage, acidity was associated with depressed feed intake

in young cattle in one study (Thomas and Wilkinson, 1973). Later work by Thomas and Wilkinson (1975) showed increased DM consumption by young calves by partial neutralization of corn silage with sodium bicarbonate. The added bicarbonate significantly increased blood pH, plasma bicarbonate and blood base excess, suggesting that acid-base balance was involved in the control of voluntary intake in young calves given corn silage as the major dietary ingredient. However, Geasler (1970) found no correlation between corn silage lactic acid content and silage intake in sheep.

Geasler (1970) reported a highly negative relationship between water soluble N content of corn silage and DMI. Thus it was felt that products arising from proteolysis in corn silage in some manner inhibit silage consumption. Various procedures to depress proteolysis in ensiled corn plant material were utilized to study the effect of water soluble N on feed intake. Wilkinson et al., (1976a) froze freshly chopped corn plant material. This reduced the water soluble N and organic acid content by 50% and 80% respectively. Dry matter intake by calves of the frozen plant material was not different from the control ensiled material. However, liveweight gain and efficiency of feed conversion were greater ($P < .05$) with the frozen material. Addition of acids (lactic and acetic acids) at the time of feeding reduced intake with no effect on liveweight gain and efficiency of feed conversion. Bergen et al., (1974), rapidly dried chopped corn plant material to 52% and 84% DM before ensiling. Although

the drying decreased organic acid production and proteolysis, DMI by sheep of the control corn silage (32% DM) and the 52% and 84% silages were not different. A direct role of proteolysis on silage intake has thus not been demonstrated. If proteolysis in corn silage is associated with reduced consumption or reduced efficiency of utilization of N, then supplementation of ensiled corn plant material with NPN may be less beneficial than of material which has not been ensiled because of the higher proportion of N in silage in water-soluble form. However, Thomas et al., (1975a,b) demonstrated that neither the source nor the site of addition of supplementary N significantly affected liveweight gain, DMI, organic matter intake or utilization of DM and organic matter by young cattle fed corn silage-based rations. McClure et al., (1972) found little difference in animal performance or efficiency of feed utilization between the addition of urea at ensiling or feeding but Geasler (1970) achieved higher liveweight gain with beef cattle when addition was made at ensiling.

It has been claimed that water soluble nitrogen is rapidly converted to $\text{NH}_3\text{-N}$ in the rumen and may hence be inefficiently utilized by the ruminal microbiota. Bergen et al., (1974) and Buchanan-Smith and Yao (1978) studied the rate of $\text{NH}_3\text{-N}$ release from corn silage water soluble N in vitro and in vivo respectively. Both studies indicated that when compared to urea, $\text{NH}_3\text{-N}$ production from water soluble N is extremely slow and may in fact limit ruminal microbial fermentation in animals fed solely corn silage. Bergen et al., (1974) further showed

that the ability of ruminal micro-organisms to utilize water soluble N from untreated or NPN-treated corn silage was not different.

The digestibility of the water insoluble N of freshly cut, untreated or NPN-treated corn silage was examined with an in vitro pepsin-pancreatin digestion system (Bergen et al., 1974). The results were 47.2%, 27.6% and 33.8 - 36.1% for freshly cut, untreated or treated corn silage respectively. The water insoluble N fraction of corn silage is likely composed of kernel protein (zein) which is not readily degraded and may bypass the rumen (McDonald, 1954). The unfavourable amino acid composition (low in lysine and high in leucine) (Bergen et al., 1974) and the low in vitro digestibility indicate a low protein quality and poor utilization of water insoluble N in the small intestine.

Although apparent N digestibility of untreated silage is lower than for NPN-treated corn silage, apparent DM digestibilities are often not different (Bergen, 1975). The increased N digestibility may be due to $\text{NH}_3\text{-N}$ lost from the rumen. Since digestibility coefficients are independent of time (i.e. rate of digestion) a lack of differences in DM digestibility between N supplemented and unsupplemented corn silage does not present a complete picture. Indeed NPN treatment or protein supplementation at feeding time has markedly improved performance of animals fed corn silage (Thomas et al., 1975a,b; Huber et al., 1968). In corn silage therefore, the primary problem is one of lack of N availability (as well as a

relatively low protein content) while for hay crop silage, often excessive N availability to the rumen has been noted. The proposal that the low N availability (% total N) may limit utilization of corn silage does not clarify the report of Dinius et al., (1968) who showed that voluntary intake by cattle of freshly chopped whole corn plant material adequately supplemented with protein was higher than the voluntary intake of cattle of the adequately supplemented ensiled chopped corn plant material. It would appear that further work is needed to evaluate the role of specific water soluble N compounds (especially non-volatile amines) on corn silage intake and performance of ruminants and to delineate the process of ammonia generation from water soluble N in the rumen.

Non-Protein-Nitrogen Treatment of Corn Silage

It has been effectively demonstrated that the fermentation process per se is an efficient one in terms of DM and energy recovery (Ruxton et al., 1975; McDonald and Edwards, 1976). The process is not however fully efficient and energy losses during the ensiling process in the form of heat and carbon evolution can be extensive (Ruxton et al., 1975).

Previous research has shown that a large share of these losses are due to the action of plant and microbial cell respiration (Woolford, 1972; Ruxton et al., 1975) and have been referred to as 'unavoidable losses' (Barnett, 1954). It is possible to control respiration loss by the addition of organic

acids or formaldehyde; however these treatments have resulted in a suppression of primary fermentation and reduction of lactic acid production (Huber et al., 1972; Britt et al., 1975). Recent studies indicate that an ammonia-molasses-mineral solution (Pro-Sil) applied at the time of ensiling can greatly reduce losses reflected by carbon dioxide evolution while stimulating normal fermentation and lactic acid production (Huber, 1975). Honig and Zimmer (1975) reported an increase of 1.6% DM in the content of lactic acid of Pro-Sil-treated silage compared to urea-treated or control silage. Huber and Santana (1972) found that ammoniated silage had higher lactic acid and water insoluble-N than urea or control silage. Increased silage ammonia content resulting from hydrolysis of urea or the addition of Pro-Sil to silage exerts a buffering action during fermentation resulting in increased levels of organic acids.

Much less is known about insidious energy losses which occur when oxygen is reintroduced into the silage mass, i.e. secondary fermentation which occurs during the feeding process. These losses have not been well characterized either microbiologically or chemically and not until recently have secondary fermentations been examined. Silages particularly susceptible to aerobic deterioration are those made from WSC-rich fodders, such as maize and those retaining high levels of residual WSC because of restricted fermentation (Ruxton et al., 1975).

Britt and Huber (1975) showed that Pro-Sil in addition to

supplying NPN, can be used to inhibit fungal growth and increase stability of silage when exposed to air. Henderson (1975) reviewed some of the effects of Pro-Sil on secondary fermentation and reported that the lactic acid content of the treated silage remained unchanged for 8 days while the untreated silage lost 96% of its lactic acid content during the same period. Juengst et al., (1975) found that Pro-Sil had a profound effect on carbon dioxide evolution during primary and secondary fermentation. Pro-Sil also eliminated yeasts and molds during primary fermentation. Bothast et al., (1973) described a similar effect of NH_3 on killing molds on high moisture corn.

Soper and Owen (1977) studied the effects of Pro-Sil on the preservation and stability of chopped whole corn plant (32% DM) when ensiled, then removed from the silo and exposed to air. Treatment of chopped corn with Pro-Sil resulted in lower loss of dry matter and increased preservation of crude protein. Based on quality measurements such as lactic acid, titratable acidity, freedom from observable mold, DM preservation and temperature stability, the treated silage was of higher quality than untreated silage after both had been exposed to air for 48h. Honig and Zimmer (1975) reported slight advantages of Pro-Sil over urea with respect to silage quality, fermentation losses and stability after opening the silo. The increased stability is due to an antifungal action of ammonia or the ammonium salts of the organic acids formed during fermentation (Britt and Huber, 1975).

Treatment of silage with urea or Pro-Sil exerts a protein sparing effect on natural protein or promotes the synthesis of bacterial protein (Owens et al., 1970; Cash et al., 1971; Huber et al., 1979). Incorporating urea into silage at ensiling masks the undesirable taste of urea and spreads the consumption over an entire day rather than in meals (Chalupa, 1970). Approximately 50% of the urea added to corn silage is hydrolysed to ammonia which exerts a buffering action during fermentation resulting in increased levels of organic acids particularly lactic acid (Huber, 1975). Henderson et al., (1971) accounted for essentially all of the Pro-Sil applied to corn silage at ensiling by increases in crude protein. The combination of elevated NH_3 and organic acids to form ammonium salts may be an asset. Ammonium salts have resulted in superior animal performance and N retention when compared to urea (Varner and Woods, 1975) and SBM (Dutrow et al., 1974).

Milk yields of cows fed NPN-treated silages have equalled or bettered those fed isonitrogenous rations from all natural protein. Milk yield data from five such studies (Huber et al., 1968; Polan et al., 1968; Huber and Thomas, 1971; Huber et al., 1973; Knott et al., 1972), showed slightly higher persistencies for cows fed the NPN-treated silages than for natural protein controls. In four studies (Lichtenwalner et al., 1972; Huber and Thomas, 1971; Huber et al., 1968; Huber et al., 1973), milk yields of individual cows producing over 29 Kg/day were compared to see if high producers on NPN-treated silage responded less favourably than high producers fed all natural

protein. There was no difference in milk yields due to form of nitrogen. Even though DM intakes were higher on the NPN silages, conversion of feed DM to milk was the same for both groups.

Increasing dietary crude protein from 8.5 to 10.5% with 0.5% urea added to corn silage greatly increased milk yields and DM intakes, but performance was best when soybean meal was added to the concentrate fed with urea-treated silage to make the total ration 12.5% CP (Huber and Thomas, 1971). The increase in water insoluble nitrogen resulting from ammonia treatment (Huber, 1975) should allow for feeding higher levels of urea in concentrate than possible with urea-treated silages. Data from three trials (Huber, 1975) suggest that cows fed ammonia-treated corn silage can tolerate more total NPN than those on urea-treated silages. Milk yields of cows receiving ammoniated silage were maintained higher than those of cows on urea silage when 1.4 to 1.5% urea was added to concentrates fed with both silages.

Huber (1975) reported a decrease in the cell wall content and acid detergent fiber levels in ammonia-treated corn silage. DM digestibility was not markedly altered but digestibilities of cell wall contents and acid-detergent fibre were depressed. Honig and Zimmer (1975) reported higher digestibilities of all nutrients in Pro-Sil treated corn silage than in untreated silage. The digestibility of crude protein was increased by 8%.

In summary, incorporation of urea or ammonia into corn silage has resulted in as high milk yields as SBM controls.

Ammoniated silage appears slightly superior to urea silage, particularly when rations are high in total NPN, apparently because of higher water insoluble nitrogen content in ammoniated silage. Non-protein-nitrogen treated silage, particularly Pro-Sil-treated, is more stable than untreated silage after the silo is opened for feeding.

Formaldehyde as a Silage Chemical Preservative

Interest in chemicals to restrict total fermentation in the silo has increased in recent years. This has arisen from both the difficulties in achieving effective selective suppression of clostridia and increasing evidence that the feeding value of silage may be reduced by the products of fermentation (Wilkins et al., 1971; Wilkins and Wilson, 1971). The application of formaldehyde has been used successfully to protect dietary casein from degradation in the rumen (Ferguson et al., 1967) and increased wool growth by sheep receiving such diets has been reported (Hemsley et al., 1973; Ferguson, 1975). Recently formaldehyde has been used as an additive during the ensiling of grasses and legumes. Addition of formaldehyde reduced anaerobic fermentation by partial sterilization of the crop with an associated depression in protein breakdown during ensiling and increased the intake of silage by sheep (Barry et al., 1972; Valentine and Brown, 1973; Wilkins et al., 1974a).

Application of formaldehyde to lucerne at 3.2% of the DM inhibited fermentation but reduced the DM intake of silage by

sheep (Brown and Valentine, 1972). However application at 0.9% of the DM inhibited fermentation and increased intake and wool production by sheep compared with untreated silage (Valentine and Brown, 1973). Formaldehyde application to fresh S.24 perennial ryegrass at the rate of 6.4 l/ton fresh weight equivalent to 6g HCHO/100g CP markedly influenced the pattern of anaerobic fermentation during the ensiling process (Beever et al., 1977). The high residual water soluble carbohydrates content of the formaldehyde treated silage, even after 90 days of anaerobic storage and the low concentration of organic acids were in agreement with results obtained by Wilkins et al., (1974a) for similar forages. Beever et al., (1977) reported relatively similar total amino-N content for untreated and formaldehyde treated silage. Whittenbury et al., (1967) observed a breakdown of 50 - 60% of the protein in crops ensiled directly and Barry et al., (1973) have shown a marked reduction in proteolysis with the addition of formaldehyde prior to ensiling. Drying the formaldehyde treated silage resulted in an increase in the concentration of N and cellulose in the DM while the content of water soluble carbohydrates was reduced (Beever et al., 1977). Formaldehyde application at the time of ensiling generally does not improve energy recovery from storage (Waldo, 1977).

The voluntary intake of silages prepared with formaldehyde alone has been found to depend on the rate of application of additive (Brown and Valentine, 1972; Wilkins et al., 1974a). In experiments of Barry et al., (1973) and three of six

experiments performed by Wilkins et al., (1974a) silages prepared with formaldehyde proved more palatable than those without additive. Large amounts of formaldehyde (> 13g/Kg fresh grass) have decreased intake considerably (Wilkins et al., 1974a; Brown and Valentine, 1972). With more moderate levels of application, 8g/Kg (0.43g HCHO/g N) to a cocksfoot-clover mixture, Barry et al., (1973) obtained increased DM intake and liveweight gain in sheep, compared with untreated silages. At lower levels of usage, 2.5g/Kg (0.2g HCHO/g N) Wilkins et al., (1974b) recorded stimulation of clostridial activity. Valentine and Radcliffe (1975) reported increased DM intake by dairy cows fed formaldehyde treated silage compared with untreated silage. Generally, average digestibility of energy in direct-cut silage was not changed by formaldehyde treatment (Waldo, 1977).

The interest attracted by formaldehyde as a silage additive is due not only to its capacity to inhibit fermentation, but also to the protection it affords against the degradation of protein during storage and in the rumen. Formaldehyde preparation of silage partly protected protein from microbial degradation during digestion in vitro with rumen liquor (Brown and Valentine, 1972) and resulted in more efficient utilization of digestible N for wool production (Valentine and Brown, 1973). In some experiments, the protection of protein by formaldehyde has been evident from the superior retention of N (Waldo et al., 1973a; Wilkins et al., 1974a), and an increase in milk production (Valentine and Radcliffe, 1975); in

some it has been revealed by a decrease in the ammonia concentration in the rumen (Barry and Fennessey, 1973; Saue et al., 1972; Wilkins et al., 1974a).

Use of formaldehyde in ensiling has resulted in a greater entry of amino acids into the small intestine (Beever et al., 1974a; Hemsley et al., 1970). Beever et al., (1977) reported results which indicated that formaldehyde application at the rate of 6g/100g CP increased total amino-N absorption by 13% but depressed overall availability of amino acids flowing at the duodenum from 75% to 67%. Protein digestibility has been decreased by formaldehyde in many studies (Ettala et al., 1975b; Barry and Fennessey, 1973; Brown and Valentine, 1972; Waldo et al., 1973a; Valentine and Brown, 1973; Wilkins et al., 1974a). The influence of formaldehyde in the rumen was more clearly apparent in animals fed on dried forages (Barry, 1971; Hemsley et al., 1970) or casein (Barry, 1972; Ferguson et al., 1967; MacRae, 1970) treated with formaldehyde than in animals offered silage.

Barry and Fennessey (1973) and Honig and Rohr (1973) observed that formaldehyde raised the acetate: propionate ratio in the rumen. But Beever et al., (1977) reported no significant differences in total VFA production when untreated, fresh and dried formaldehyde-treated silages were fed to sheep. On the untreated silage only 56% of the energy apparently digested in the rumen was converted to VFA energy whilst a mean value of 74% was recorded for the other 2 diets. The inefficient conversion in the rumen of sheep fed the untreated

silage was similar to that measured previously by Beever et al., (1974b) for sheep fed fresh and frozen ryegrass. This may be due to extensive degradation of dietary protein in the rumen and the associated inefficiencies of this process. Using the results of Baldwin et al., (1970), Beever et al., (1977) calculated that heat and methane production resulting from protein degradation was likely to have been at least 4 times higher on the untreated silage than on the treated silages which would have been sufficient to account for at least 1/3 of the difference observed in energy loss.

Recently the expression of microbial efficiency in terms of microbial mass synthesized/mole of ATP (Y_{ATP}) produced during rumen fermentation has been adopted by several workers (Smith, 1975). In the study of Beever et al., (1977), Y_{ATP} values of 16.6, 5.0 and 5.6 were obtained for untreated, fresh and dried formaldehyde-treated silage respectively. The value of 16.6 for animals on untreated silage was within the range quoted by Hogan and Weston (1970). However the values for fresh and dried formaldehyde treated silage were much lower than are generally accepted. The availability of ammonia to the microbes in the rumen of sheep fed the treated silages was lower than for the untreated silage and may have restricted protein synthesis and the presence of free formaldehyde may also have had some effect on microbial activity and growth (Wilkins et al., 1974a). Uncoupled fermentation, during which degradation can proceed but microbial protein synthesis is restricted, could also explain the reduced yields

of microbial protein in sheep fed the treated silages (Beever et al., 1977).

The addition of formaldehyde at low rates can induce a clostridial fermentation so that the scope of formaldehyde alone as a silage additive is limited (Wilkins et al., 1974b). Formaldehyde is effective as a fermentation inhibitor, particularly when combined with acids and limits breakdown of the protein fraction of grass (Wilkins et al., 1974b). Complete suppression of fermentation was obtained with formaldehyde applied at the rate of 5 l/ton fresh weight plus formic acid at 2 l/ton (Wilkins et al., 1974b). Ettala et al., (1975a) obtained good quality silage when additives containing formaldehyde and formic acid were applied at rates of 4 - 6 l/ton. Donaldson and Edwards (1977) obtained similar results with wilted ryegrass silage. Generally, recoveries of energy from storage have been improved 3% by treatment with formic-formaldehyde mixtures (Waldo, 1977).

Ettala et al., (1975b) found no significant differences in DM intake when silages prepared with acid additives and formic-formaldehyde mixtures were fed to lactating dairy cows. Donaldson and Edwards (1977) reported similar DM intake by sheep fed untreated or formic-formaldehyde treated wilted ryegrass. Barker et al., (1973) ensiled alfalfa-bromegrass with or without a mixture of formaldehyde and formic acid. There was no significant difference in DM intake by dairy cows between silages. The average intake of direct-cut silage when fed to growing heifers without concentrate was

increased by 46 Kcal DE/KgW^{3/4} by formic-formaldehyde mixture (Waldo, 1977), although in one direct comparison intake increased by 29 Kcal DE/KgW^{3/4}.

Donaldson and Edwards (1977) reported no effect of formic-formaldehyde mixture on digestibilities of organic matter and dry matter in sheep. Treatment with formic-formaldehyde mixture depressed N digestibility in lactating cows (Ettala et al., 1975b) and growing heifers (Waldo et al., 1973a). Ettala et al., (1975b) reported no improvement in N balance by sheep fed silages made with formic-formaldehyde mixture. Waldo et al., (1973a) obtained significant increases in N balance and daily gain in growing heifers fed formic-formaldehyde treated grass-legume silage. The average daily gains from feeding trials as summarized by Waldo (1977) were increased 0.36 Kg by formic-formaldehyde mixtures. Milk yield has not been affected by feeding formic-formaldehyde treated silage (Barker et al., 1973; Ettala et al., 1975b). Although the formic-formaldehyde mixture decreased milk production 2.2 Kg below that of untreated direct-cut silage, five direct comparisons produced essentially equal milk on formic acid and formic-formaldehyde mixtures (Waldo, 1977).

Silage prepared with paraformaldehyde has been found equal in palatability to silage treated with formic acid (Waldo et al., 1973b; Waldo and Keys, 1974). Waldo et al., (1975) obtained similar daily gains in heifers fed with silages treated with 0.5% paraformaldehyde or 2.5% formic acid which were better than for untreated silage. These gains resulted from

improved intake and feed conversion. These results were consistent with the animal response in experiments where formic-formaldehyde mixtures were used (Waldo et al., 1973a). From experiments on nitrogen supplementation of silages that give small gains (Waldo et al., 1973b), limiting protein degradation during ensiling is apparently the major factor contributing to improved gains on treated silages. Formaldehyde either alone, in mixture with formic acid or as paraformaldehyde appears as effective as formic acid in limiting the protein degradation.

Protein Solubility of Ruminant Feeds

High solubility of crude protein in the rumen has been blamed as a factor in promoting inefficient utilization of protein by ruminants. Soluble nitrogen in purified proteins is positively correlated with the degradation of nitrogenous material in the rumen (Blackburn, 1965; Henderickx and Martin, 1963). Insoluble protein therefore has a greater chance of escaping the rumen and reaching the lower gut where it can be efficiently digested and absorbed. This concept has led to development of nitrogen solubility procedures for evaluation of ruminant feeds. Much of this work has been with solvents differing in chemical and physical properties, resulting in a variety of solubility values for each feed (Evans and Biddle, 1971; Little et al., 1963; Peter et al., 1971; Tagari et al., 1962; Sharma et al., 1972; Wohlt et al., 1973; Henderickx and Martin, 1963; Aitchison et al., 1976).

These variations demonstrate the need for a repeatable and accurate method of determining protein solubility representative of protein degradation in the rumen.

Although it is important to recognize the specific properties of rumen fluid as a protein solvent, Jancarik and Proksova (1970) found that autoclaved rumen fluid could cause variations in protein solubility measurements. Henderickx and Martin (1963) reported that degradation of purified proteins during 6h in vitro incubations in rumen fluid was highly correlated with the proteins' solubilities in Wise Burroughs (Burroughs et al., 1950) mineral mixture diluted to 10% with distilled water. However, they did not compare solubilities in the mineral buffer to solubilities in autoclaved rumen fluid.

Wohlt et al., (1973) determined the effects of pH, extraction time, processing and solvent type (autoclaved rumen fluid or Wise Burroughs mineral mixture) upon protein solubility. Solubility did not differ between solvents with 60 min. extraction time and at pH 6.5 and 7.5. Feeds were grouped as to major protein fractions and amount of processing. Feeds with protein fractions composed mainly of albumins and globulins had a higher solubility than those composed primarily of prolamins and glutelins. Heated feedstuffs (soybean meal, cotton seed meal, feather meal) had moderately low solubilities indicating that heating and processing markedly affect solubility.

The amount of nitrogen extracted from purified proteins

by Wise Burroughs mineral mixture (BMM) has been closely correlated with the solubility of these proteins in autoclaved rumen fluid (ARF) (Wohlt et al., 1973). However BMM is complicated to prepare and has a short shelf-life and autoclaved rumen fluid is variable in composition and difficult to obtain (Crooker et al., 1978). Therefore it would be desirable to find a solvent which had the ability of BMM and ARF to solubilize proteins but without their disadvantages. Crooker et al., (1978) found that the quantity of nitrogen extracted by either a modified BMM or McDougal's artificial saliva differed from that extracted by ARF, whereas that extracted by either BMM or by sodium chloride solutions did not differ. Changing ionic strength of the solvents had no significant effect on the quantity of nitrogen extracted, contrary to the observations that ionic strength affects protein solubility (Lehninger, 1975; Salobir et al., 1969).

The solubility and kinetic studies of Pichard and Van Soest (1977) demonstrated that there were four general categories of nitrogen in ruminant feeds. These include a water-soluble NPN fraction A which includes nitrate, ammonia and amines and insoluble fractions which include a rapidly degradable protein fraction B₁, a more slowly degradable fraction B₂ and an unavailable fraction C. Fermentation of the nitrogen fraction in forages increases A and C fractions at the expense of the B fraction such that in badly damaged silages nitrogen is distributed mainly as NPN and unavailable nitrogen. The high but variable protein solubility in

fermented feedstuffs is attributed to proteolysis by plant enzymes and protein solubilization by acid during storage (Bergen, 1975).

A number of comprehensive systems have been proposed for evaluating protein nutrition of ruminants (Burroughs et al., 1975; Miller, 1973; Roy et al., 1977; Satter and Roffler, 1975). In each of these systems, consideration has been given to degradation of dietary protein in the rumen. The amino acids absorbed across the small intestinal wall are assumed to be derived from either microbial protein or dietary protein which bypassed the rumen undegraded. The quantities of amino acids available for metabolic functions are calculated from the respective amino acid profiles of the microbial and dietary protein sources.

For this method of protein evaluation, it is necessary, due to lack of data, to make the assumption that the amino acid profile of the bypass protein is the same as originally ingested. For this to be true, all amino acids in a given protein source would have to be degraded in the rumen to the same extent. The question arises as to the division of the individual amino acids between the soluble and insoluble protein fractions which may or may not be composed of different proteins. MacGregor et al., (1978) studied, in vitro, the amino acid profiles of total and soluble protein in common feedstuffs. In the majority of feedstuffs analyzed, there were marked differences between the amino acid profile of the total protein and the amino acid profile of the insoluble

protein fraction. This suggests that the amino acid profile of the undegraded protein which bypasses the rumen may be different from the amino acid profile of the dietary protein as originally ingested. If these differences exist in vivo, as suggested by Sniffen and Hoover (1978), they will be of consequence in the application of several recently proposed systems of protein evaluation which assume no differences in amino acid profile between total and undegraded feed protein.

Previous work has indicated a positive correlation between solubility of protein in a dilute mineral mixture and the extent of protein degradation of that protein in the rumen (Dingley et al., 1975; Hawkins and Strength, 1977; Mertens, 1977; Sniffen, 1974; Wohlt et al., 1976). Owens (1978) reviewed extensively different systems to predict rumen bypass of protein. In vitro techniques used to measure ruminal degradation have generally been based on ammonia release from the protein when incubated with ruminal liquor. Although ammonia is the primary end-product of ruminal protein degradation, approaches of this type may lead to inaccuracies for at least two reasons (Broderick, 1978): (i) Ammonia accumulation as an index of degradation is complicated by the fact that its uptake for microbial growth will reduce estimates of degradation; (ii) Simple accumulation of protein degradation end-products does not take into account rate of ruminal passage of the protein, which, along with degradation rate, is also a determinant of ruminal protein escape. The above observations are supported by Little et al.,

(1963) and Crooker et al., (1978) who found no consistent relationship between soluble nitrogen and free ammonia from rumen fluid incubations with various feedstuffs. Differences in the amount of fermentable carbohydrate in each feedstuff probably contributed to the lack of a consistent relationship between soluble nitrogen and ammonia accumulation since several investigators (Annison et al., 1954; Lewis, 1962; Johnson, 1976; Vasilatos et al., 1976) have shown that carbohydrates can play an important role in ruminal ammonia formation and utilization. Broderick (1978) developed an in vitro procedure for casein, in which rates of ruminal protein degradation and proportions of amino acid escaping ruminal degradation were estimated from the release of amino acids plus ammonia in the presence of hydrazine sulfate. The method may require some modification before application to more complex feed proteins of varying solubilities.

Rations can be formulated for different protein solubility from commonly used natural ingredients (Wohlt et al., 1976). Aitchison et al., (1976) in three nitrogen balance trials with lactating dairy cows, found that the utilization coefficient of insoluble nitrogen was greater than for soluble nitrogen. Dingley et al., (1975) reported that amino acid supply to the udder was influenced by solubility of dietary proteins. Majdoub et al., (1978) observed no effect of soluble nitrogen on average daily intake of DM, crude protein or net-energy-lactation of dairy cows. However, the lower nitrogen solubility (22 vs 42%) increased milk yield but milk composition

was not affected. Hawkins and Strength (1977) reported no significant difference in milk yield but significantly ($P < .01$) higher total solids for cows fed diets containing the higher soluble N (30.7 and 42.5%) than for those fed diets containing the lower soluble N (28.7%). Wohlt et al., (1976) found that water consumption, urine volume, urinary N excretion, rumen ammonia and butyrate concentrations were higher ($P < .01$) for wethers receiving rations in which 35% of dietary protein was soluble compared to 13% solubility.

Prigge et al., (1978) reported no difference in abomasal passage of feed nitrogen from corn grain (dry, steam flaked and high moisture) with protein solubilities of 12, 8 and 64% respectively. Apparently the protein in fermented grain, though solubilized, bypassed ruminal digestion. This finding casts doubt on the value of simple protein solubility as a predictor of rumen bypass.

The potential exists for improving protein utilization in lactating dairy cows by formulating rations according to nitrogen solubility. As pointed out by Henderickx and Martin (1963), the protein in solution is more accessible to the microbial activity and this is the fundamental principle in determining protein solubility in feedstuffs. Use of nitrogen solubility in formulating dairy rations could reduce the absolute quantity of crude protein required and reduce costs. Presumably, low N solubility results in less degradation of protein by ruminal micro-organisms thereby producing a different amino acid profile in the lower gastrointestinal tract.

Degradation of Nitrogen in the Rumen

Nitrogen enters the rumen in food, mainly as protein or in silage as protein and amino acids, in saliva as urea and possibly by diffusion of urea across the rumen wall (Nolan et al., 1973). As judged from the N content of the material flowing at the duodenum in sheep, endogenous additions vary with the level and type of feeding from about 1 to 9g N/day (Weston and Hogan, 1968; Nicholson and Sutton, 1969; Egan, 1974; Thomas et al., 1976). The main nitrogenous end-product of degradation in the rumen is ammonia although peptide and amino acid intermediates are formed from proteins and purine and pyrimidine bases from nucleic acids (Smith, 1975).

Several authors (Allison, 1970; Blackburn, 1965; Bryant, 1970; Leng, 1973) have summarized information on microorganisms responsible for proteolysis and the nature of microbial proteases. Rumen bacterial proteases are cell bound but are located on the cell surface to provide free access to substrate and are comprised of both exo- and endo-peptidase. The metabolic importance of protein degradation in the rumen may be to supply ammonia and nonammonia nitrogenous nutrients. Ammonia is the primary nitrogenous nutrient for rumen bacterial growth (Al-Rabbatt et al., 1971; Nolan and Leng, 1972; Mathison and Milligan, 1971), although some species of bacteria use peptides directly for protein synthesis (Allison, 1970). Proteolytic protozoa digest bacterial protein which is the major source of amino acids for growth of these microbes (Allison, 1970).

Ruminal degradation of amino acids have been reviewed by several authors (Allison, 1970; Armstrong and Hutton, 1972; Bryant, 1970; Leng, 1973). Deaminative activity occurs less frequently in rumen bacterial strains than does proteolytic activity. The primary function of deamination may be for the production of branched fatty acids which are required growth factors for some strains of rumen bacteria (Allison, 1970; Bryant, 1970).

In spite of proteolytic capabilities of rumen microbes, substantial amounts of ingested protein are resistant to degradation and bypass the rumen. Data summarized by Chalupa (1975) indicate that as little as 40% or as much as 80% of the dietary protein normally might be degraded in the rumen and that there can be wide differences among feed ingredients in the extent of ruminal degradation of the protein fraction.

Two of the factors contributing to differences in apparent ruminal degradation of proteins are solubility of protein in rumen fluid (Annison, 1972; Wohlt *et al.*, 1973) and length of time protein is retained in the rumen. Solubility is an inherent characteristic of proteins which can be modified by procedures discussed elsewhere in this review. Ruminal degradation of proteins can be diminished by decreasing retention time in the rumen. Rate of passage of digesta is influenced by food intake, specific gravity, particle size of diet, concentrate to roughage ratio and rate of rumen digestion (Balch and Campling, 1965).

Dietary NPN as well as urea from saliva and urea from

the rumen wall are degraded into ammonia and carbon dioxide (Chalupa, 1972). Rumen contents possess high urease activity; this is in contrast to biurataase and uricase that must be induced by feeding the respective NPN compounds (Chalupa, 1972; Oltjen et al., 1968; Devlin and Woods, 1965).

Synthesis of Microbial Protein in the Rumen

Bacterial yield or output from the rumen is a function of bacterial concentration or population and rate of growth or turnover. Recent reviews (Bryant, 1973; Smith, 1975) have discussed the nutrients and other factors limiting bacterial population. Nitrogen limitation (Satter and Slyter, 1974) and lack of sulfur, other minerals and certain branched-chain fatty acids also can limit bacterial populations under specific conditions (Allison, 1970; Bryant, 1973). But the more typical limitation is energy supply. Anaerobiosis of the rumen severely limits ATP production. From 1 mole of hexose, bacteria anaerobically can generate only 3.6 - 5.6 moles ATP, depending on the ratios of specific end-products formed (Baldwin, 1970; Isaacson et al., 1975).

The mass of bacteria that will be produced per mole of ATP has been termed the Y_{ATP} or yield ATP (Bouchop and Elsdén, 1960). This Y_{ATP} was initially estimated at 10g dry cells/mole of ATP (Bouchop and Elsdén, 1960). More recent research (Isaacson et al., 1975; Stouthamer and Bettenhausen, 1973) questions whether Y_{ATP} is indeed a constant or a variable as it appears to range from 5 to 20 under ruminal conditions.

This means that for every 100g organic matter fermented in the rumen, gm of cells produced may range from 9 to 37 (Smith, 1975; Stouthamer and Bettenhausen, 1973).

Owens and Isaacson (1977) gave the following reasons for variation in Y_{ATP} : (i) Chemical composition of bacterial cells may influence yield. The accumulation of ash or starch would increase the total mass of bacteria and dilute protoplasm and protein; (ii) Transfer of metabolic intermediates between species appears to enhance yields (Reichl and Baldwin, 1976); (iii) Availability of cell components may influence yields; (iv) Energy expended by bacteria for cell maintenance and replacement of lysed cells may influence yields.

At high ruminal dilution rates, the ATP needs for maintenance are quite low and much of the ATP is available for cell synthesis (Bergen and Yokoyama, 1977). Isaacson et al., (1975) reported that microbial yield from a continuous flow system is dependent upon growth rate of bacteria or dilution rate. Studies by Cole et al., (1976) indicated a strong relation between rumen turnover or dilution rate and microbial protein synthesis. Kropp et al., (1977a,b) and Prigge et al., (1978) obtained similar results, indicating that turnover rate of rumen contents is an important factor in efficiency of microbial protein synthesis.

If energy is adequate, then other factors may limit microbial growth. It is generally recognized that ammonia is quantitatively the most important nitrogenous nutrient for rumen bacteria (Allison, 1970; Bryant, 1970) and the rate of

microbial growth appears to increase with ammonia concentration up to about 5 mg/100 ml, when it reaches a maximum (Satter and Slyter, 1974). Many species of rumen bacteria synthesize protein from ammonia and some use ammonia preferentially. Others have a preference for preformed amino acids although these may be present as peptides before they are efficiently utilized (Allison, 1970). Sulfur intake may sometimes be limiting if ruminants are given diets containing large amounts of NPN. The studies of Bouchard and Conrad (1973b) and Chalupa et al., (1973) generally confirmed earlier reports that the requirements of the rumen micro-organisms are met if the diet has an N:S ratio no greater than ten. Protozoa require both amino acids and pyrimidine bases which they derive from the diet or from engulfment of bacteria (Coleman, 1975).

The utilization of N in the rumen has recently been studied using ^{15}N (Nolan, 1975). The results, mainly from forage diets, show that 50 to 70% of bacterial nitrogen and 31 to 55% of protozoa nitrogen is derived from ammonia. Thus the uptake of preformed amino acids makes a substantial contribution to microbial synthesis. The extent to which this is necessary to maintain synthetic efficiency is, however, difficult to assess. Deficiencies in the supply of valine, leucine, isoleucine, phenylalanine and tryptophan to the microbes have been reported with diets containing little protein and a high proportion of NPN (Thomas, 1973). However, only the results of Hume (1970) indicate that the mixture of

amino acids supplied by dietary protein has an influence on microbial synthesis.

The results of Nolan (1975) also indicate the significance of engulfment of bacteria by protozoa. About 20% of the nitrogen incorporated into microbes was recycled through the ammonia pool presumably due to protozoal activity or lysis of bacteria. With cereal grain diets where the numbers of protozoa are high, engulfment of bacteria could account for about 9g N/day in the sheep (Coleman, 1975) and since a large proportion of the protozoa are sequestered in the rumen (Weller and Pilgrim, 1974) recycling of N could be extensive.

Protein synthesis is also influenced by the microbial population and conditions in the rumen. These factors were highlighted by the finding that in sheep given a high-concentrate diet, the efficiency of protein synthesis was directly correlated with the molar proportion of propionate in the rumen (Ishaque et al., 1971). Similar results have also been obtained under other dietary conditions (Thomas, 1973). Subsequently, it was shown (Hodgson and Thomas, 1972) that with the diet used by Ishaque et al., (1971), the molar proportion of propionate was inversely correlated with the clearance rate of the rumen liquid phase. This relationship has now been confirmed to operate with a wide range of mixed forage and concentrate diets (Harrison et al., 1973; Hodgson et al., 1976) although it does not apply with moderate or poor quality forages (Thomas, 1977). But it is now clear that protein synthesis is not always correlated with the proportion of

propionate (Chamberlain et al., 1976) and that where correlations occur they can be positive or negative (Harrison et al., 1976; Kennedy et al., 1976). In the rumen, variations in clearance rate influence not only microbial metabolism but also the composition of the bacterial population and the ratio of bacteria to protozoa (Latham and Sharpe, 1975; Potter and Dehority, 1973). Thus simple relationships between efficiency of protein synthesis and the clearance rate are complicated by shifts in the composition of the microbial population.

Digestion of material flowing into the duodenum provides the animal with most of the essential amino acids. Investigation of the origins of the different nitrogen compounds entering the duodenum has depended mainly upon the development and use of techniques to estimate the microbial contribution (Sutton and Oldham, 1977). These techniques all involve the determination of the concentration of a particular microbial component in duodenal digesta. The contribution of microbial N to the digesta is then calculated from a value for the ratio of the chosen component to nitrogen in the micro-organisms which contribute to the duodenal digesta.

The most commonly used markers include α - ϵ - diamino-pimelic acid (DAP) (Amos et al., 1976; Hogan and Weston, 1970; Hutton et al., 1971; Harrison et al., 1973; Lindsay and Hogan, 1972; Miller, 1973), total nucleic acids and (or) RNA (McAllan and Smith, 1971; Ling and Buttery, 1976; Kropp et al., 1977a,b; Coelho da Silva, 1972a,b), DNA (Temler-Kucharski and Gausseres,

1965), ^{35}S (Beever et al., 1974c; Hume, 1974; Leibholz, 1972), ^{15}N (Smith et al., 1975; Pilgrim et al., 1970) and ^{32}P (Smith et al., 1978; Van Nevel et al., 1975). None of these methods are, however, without error, because it is difficult to obtain a microbial sample representative of that passing from the rumen (Smith, 1975). As the DAP contents of different bacterial species vary widely (Synge, 1953; Purser and Buechler, 1966) and it is absent from protozoa, the use of this constituent is likely to be particularly sensitive to unrepresentative sampling.

The RNA: total N ratio of different bacterial species in the rumen varies relatively little and the protozoa appear to show a value similar to bacteria (Smith et al., 1975). But RNA as a microbial marker suffers from the disadvantage that although dietary RNA is rapidly degraded in the rumen (McAllan and Smith, 1973) enough contamination may remain from the diet to affect the results (Smith et al., 1978). Even an isotopic marker, incorporated from an inorganic form in the rumen, may not be uniformly distributed throughout the bacterial population. This was shown for ^{15}N by Pilgrim et al., (1970) and for ^{35}S by McMeniman et al., (1976). But with a fairly steady state, this is unlikely to be true of ^{32}P as, according to Van Nevel and Demeyer (1977), rumen bacteria obtain nearly all their phosphorus from inorganic sources.

Ling and BATTERY (1976) working with sheep and McMeniman (1975) working with cattle showed that estimates of microbial-N

at the duodenum based upon RNA measurements were higher than those based upon measuring an ^{35}S label. If the RNA based values are multiplied by a factor of 0.85 (Smith et al., 1978) then agreement with the ^{35}S based values is close.

The protozoal contribution to duodenal-N would approximately be included in estimates based upon RNA but not in those based upon DAP (Smith et al., 1978). Smith et al., (1978) concluded that on the average for the cow, about half the microbial-N at the duodenum was of protozoal origin. This was compatible with conclusions of Hagemester (1975) for dairy cows and Abou Akkada and El-Shazly (1976) for sheep based upon the flow of amino ethyl phosphoric acid (AEP) at the duodenum. Walker and Nader (1975) concluded from results based on ^{35}S and DAP methods that about 30% of the microbial-N at the duodenum of sheep was protozoal. This does not agree with the conclusions of McMeniman (1975) that Microbial-N flow at the duodenum was similar whether based upon ^{35}S or DAP, or support the view of Weller and Pilgrim (1974) that relatively little protozoal-N leaves the rumen.

As energy is usually the factor limiting microbial growth, protein synthesis has been expressed as a function of either organic matter (OM) or DM apparently digested in the rumen (Sutton and Oldham, 1977). Kropp et al., (1977b) found that microbial protein synthesis in steers ranged from 21.8 to 24.3g/100g OM apparently digested in the rumen. These results are equivalent to 22 - 23g commonly reported for sheep (Hogan and Weston, 1970; Thomas, 1973). Smith et al.,

(1978) indicated a mean value of 25g N/Kg OM apparently digested in the rumen of cows. This was within the spread of values (20 to 50g N/Kg OM apparently digested in the rumen) reported in other investigations reviewed by Smith (1975). Czerkawski (1978) reassessed published determinations of efficiency of synthesis of microbial matter in the rumen and emphasized the distinction between organic matter apparently digested but corrected for microbial matter entering the duodenum, organic matter truly digested and organic matter apparently digested. According to his analysis, the average efficiency of synthesis of microbial matter in the rumen was 19.3g N/Kg OM truly digested. The corresponding values with OM corrected for microbial matter and with OM apparently digested were 21.9 and 29.5g N/Kg OM, respectively. It is reasonable to use a generalized value of 30g N/Kg OM apparently digested in the rumen or an equivalent value of 23g N/Kg OM truly fermented in the rumen for calculating the maximum amount of microbial protein that can be digested in the rumen (Roy et al., 1977) but it must be recognized that considerable variation may occur under particular conditions.

Nutritional Value of Nitrogenous Compounds entering the Lower Gut

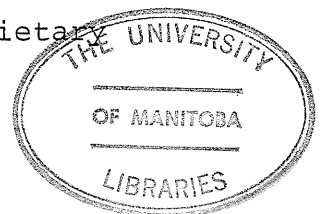
The principal nitrogenous compounds entering the small intestine of ruminants are protein (from dietary, microbial and endogenous sources), nucleic acids (mainly from microorganisms) and any ammonia that results from the microbial fermentation of nitrogenous materials in the reticulo-rumen

but is neither utilized for microbial cell synthesis nor absorbed prior to the proximal duodenum (Armstrong and Hutton, 1975). The proportion of dietary protein that reaches the proximal duodenum intact depends largely on the solubility of the protein in rumen liquor, the level of food intake and also on the processing involved in preparation of the feed (Chalupa, 1975). There are few data on the amount of endogenous protein added in the abomasum but values indicating that it might supply 7 to 25% of duodenal-N have been suggested (Miller, 1973; Smith and McAllan, 1973).

Isolated preparations of rumen bacteria and protozoa have been reported to contain 35 - 80% and 17 - 55% crude protein, respectively (Chalupa, 1972). The wide range in crude protein content is probably mainly due to the varying degree of contamination of the microbial preparations with digesta. Amino acids are present in both cell walls and cytoplasm, but because cell walls constitute only 15% of the dry weight of rumen bacterial cells, most of the amino acids are contained in non-cell wall material (Hoogenraad and Hird, 1970). Amino acids contained in cell walls may not be released by proteolytic enzymes in the abomasum and small intestine and therefore may be of limited nutritional value to the animal (Allison, 1970). Purser (1970) found a difference between the amino acid composition of protozoa and bacteria. Protozoa contain slightly higher quantities of certain essential amino acids which may suggest a superior nutritional value.

While the amino acid composition of rumen microbial protein exhibits a remarkable constancy, there are indications that the release and availability of specific amino acids may vary. Amino acid availability in rumen microbial protein has been studied by digesting individual strains or bulk preparation of bacteria and protozoa with pepsin and pancreatin (Purser, 1970; Bergen et al., 1967). Bergen et al., (1967) found differences between individual strains of bacteria with respect to protein quality. Allison (1970) suggested that these differences might be partly explained by differences in amino acid composition of bacterial cell walls. Purser (1970) indicated that amino acid release patterns must be considered in terms of the digestibility of the protein source to yield specific amino acids and also in terms of the influence of the composition of the released amino acids on the rates, patterns and extent of absorption of amino acids from the alimentary tract. Generally rumen protozoa are slightly higher in biological value than bacteria and because of high true digestibility, net utilization of protozoal protein is greater (Chalupa, 1972).

Ben-Ghedalia et al., (1974) and Orskov et al., (1971) showed that about 15 - 25% of the digesta N which disappears in the intestine of sheep, disappears in the large intestine. Some nitrogenous constituents of bacterial cell walls (DAP and muramic acid) are not removed in the small intestine of sheep but are degraded in the large intestine (Mason and Milne, 1971). Mason (1971) showed that excretion of non-dietary



fecal N was positively related to intake of truly digestible DM in steers and sheep fed medium and high quality roughages. Grinding and pelleting a grass ration for steers resulted in an increase in the excretion of non-dietary fecal N. These responses reflect the dominating effect of N of microbial residues from the rumen and the hind-gut on the excretion of bacterial and endogenous debris N and non-dietary fecal N.

Substantial amounts of nucleic acid N are produced during rumen microbial synthesis. Smith (1969) concluded that per unit of dietary N incorporated into microbial N, 80% is converted into bacterial protein and 20% is converted into nucleic acid N. Sheep and cattle digest about 75 - 90% of these nucleic acids between the duodenum and ileum (Coelho da Silva, 1972a,b; Smith and McAllan, 1971; Armstrong and Hutton, 1975) and the consequence of this to the host animal is the release of phosphorus from nucleic acids. Limited evidence suggests that 40 - 50% of the microbial nucleic acid N is either not absorbed from the gut or is absorbed and excreted as allantoin in urine (Smith, 1969). Topps and Elliott (1965) reported a significant correlation between the concentration of nucleic acids in the rumen and the excretion of allantoin in urine. Nucleic acid N may therefore be of limited value to the animal and production of nucleic acids in the rumen may result in a wastage of nitrogen.

Rumen Bypass and Protection of Proteins and Amino Acids

Amino acids absorbed from the small intestine of ruminant animals are supplied by microbial protein synthesized in the rumen, undegraded or protected food proteins and amino acids which bypass the rumen and endogenous secretions. Little can be done to influence directly the amino acids provided by the latter, but the quantity of amino acids in microbial proteins and materials which bypass the rumen can be modulated (Chalupa, 1975). The marked stimulus to wool growth and milk production produced by the abomasal infusion of protein (Clark, 1975), indicates that the yield of microbial protein is quite inadequate for maximum production.

Proteins: Protection of the protein from rumen degradation can be achieved in various ways as reviewed by Chalupa (1975) and Ferguson (1975). The natural rumen bypass method is esophageal groove closure which is a normal function in young ruminants, but occurs rarely in mature animals. Factors thought to influence groove closure include age, posture of animal while drinking, site of delivery into the esophagus and temperature and chemical composition of the liquid (Ørskov, 1972). Rumen bypass of nutrients in older animals by closure of the esophageal groove has resulted in significant improvements in growth rate and feed efficiency (Ørskov, 1972), but practical methods for stimulating the reflex have not been established.

Proteins can be modified to increase their resistance to

rumen degradation by physical and chemical means. Feed processing procedures like oil extraction can influence the magnitude of protein degradation in the rumen. Increased ruminal degradation may be the result of disruption of the protein matrix whereas heat applied or generated during grain processing can decrease ruminal degradation of protein (Hale, 1973). In addition, some processing procedures will increase microbial protein production by increasing the quantity of starch fermented in the rumen (Waldo, 1973).

The effect of heat treatment during meal manufacture in reducing the rate of microbial fermentation is attributable to reduced solubility of the protein (Tagari et al., 1962), although coarse lumps of insoluble protein may be retained within the rumen for longer periods and thereby fermented to a greater extent (Chalmers et al., 1954). Destruction of inhibitors in some protein sources, such as trypsin inhibitor in soybeans, can increase animal performance, but improvements are largely the result of decreasing ruminal degradation of proteins (Goering and Waldo, 1974). Protection produced by heating is counter-balanced by decreases in digestibility and biological value caused by the Maillard reaction between sugar aldehyde groups and free amino groups. However, if this reaction can be controlled to decrease protein solubility and degradation in the rumen without adversely affecting intestinal protein digestibility, animal performance evaluated by either nitrogen retention, weight gain or feed efficiency is increased (Danke et al., 1966; Glimp et al., 1967; Hudson

et al., 1970; Little et al., 1963).

Reduced in vivo digestibilities have been reported for low-moisture silage, high moisture-baled stacked hay and artificially dried hay as compared to digestibilities of companion direct-cut silage or sun-cured hay (Sutton and Vetter, 1971; Thomas et al., 1972; Goering et al., 1974b). The extent of heat damage can be evaluated by assaying for acid-detergent insoluble nitrogen (ADIN) (Goering et al., 1972), and is probably the result of irreversible binding or destruction of amino acids. Effective heating time, temperature and moisture were related to amount of damage in forages (Goering et al., 1973; Yu, 1976). Silage fermentation converts soluble carbohydrates to organic acids and should reduce the susceptibility of the resulting forage to heat damage (browning) since soluble carbohydrates are required for the browning reaction (Van Soest, 1965). This hypothesis was supported by Gordon (1967). Haylage (50% DM) undergoes less fermentation, retains more soluble carbohydrates and should be more susceptible to browning than direct-cut silage.

Chemical modification of dietary protein can be achieved by treating with vegetable tannins (Delort-Laval et al., 1972; Leroy et al., 1965). The possibility that tannins in seeds and forages provide some degree of natural protection has also been recognized (McLeod, 1974). Tannins have been classified as hydrolysable or condensed. Hydrogen bonding has been proposed as the most likely mechanism of reversible crosslinking of proteins with hydrolysable tannins. Under aerobic conditions

irreversible oxidative coupling occurs (McLeod, 1974). The tannin-protein complexes formed by condensed tannins are unlikely to be hydrolysed to yield amino acids in the abomasum (Zelter et al., 1970). McLeod (1974) suggested that forage tannins are unlikely to serve as a 'built-in' means of protein protection because the tannin found in forages so far are generally of the condensed type. Tagari et al., (1965) suggested a possible disadvantage of the use of tannins for protein protection, because certain tannins interfere with the cellulolytic activity of the rumen micro-organisms.

Formaldehyde was first shown to be an effective means of protecting dietary protein without rendering it indigestible in the small intestine by Ferguson et al., (1967). Other aldehydes such as acetaldehyde, glutaraldehyde and glyoxal were also effective, but appeared to possess no advantages over formaldehyde which is cheaper. Treatment of casein with formaldehyde generally has resulted in increased nitrogen retention, wool growth and muscle growth (Chalupa, 1975). While treatment of plant proteins has not yielded consistent responses, growth rates and feed efficiencies have been improved (Chalupa, 1975). Broderick and Lane (1978) reported no significant differences in milk yield and milk composition of cows supplemented with formaldehyde-treated casein compared to those supplemented with untreated casein. Similar results were obtained by Kellaway et al., (1974) and Wilson, (1970). However compared to no supplement, formaldehyde-treated and untreated casein significantly increased milk

and milk protein production (Broderick and Lane, 1978; Kellaway et al., 1974; Wilson, 1970). Kaufmann and Hagemester (1976) reported that the rate of degradation of formaldehyde-treated protein in the rumen of dairy cows was decreased by 20% as compared with untreated protein. However, the amount of bacterial protein reaching the intestine, digestibility in the intestine and lysine digestibility were unaffected by formaldehyde treatment.

Adding formaldehyde to forages at ensiling appears to promote more consistent increases in animal performance (Chalupa, 1975), and the possible reasons for this are discussed in another section. Treating proteins with formaldehyde usually increases fecal nitrogen excretion but the effect appears to be less severe with casein than with plant proteins (Chalupa, 1975). Reis and Tunks (1969) indicated that infused untreated casein was 6 - 8% more digestible than dietary formaldehyde-treated casein. Other workers (MacRae et al., 1972; Faichney and Weston, 1971) confirmed that treatment of casein with formaldehyde decreased nitrogen digestibility, but there were significantly increased amounts of nonammonia N entering and apparently absorbed in the small intestine.

Amino Acids: Various procedures have been devised to protect free amino acids from ruminal degradation. The product designed by Sibbald et al., (1968) was composed of 20% DL-methionine, 20% kaolin and 60% tristearin. Mowat and Deelstra (1972) reported increased weight gains and feed efficiencies in sheep supplemented with urea and 0.4% encapsulated methionine.

However, a marked reduction in performance was noted at the 0.6% level, indicating a toxic effect. Broderick et al., (1970) found that feeding encapsulated methionine to supply 5, 15 or 45g/day methionine to lactating dairy cattle had no effect on milk production or composition. Similar results were reported by Williams et al., (1970). Increases in plasma methionine:valine ratios (Linton et al., 1968; Broderick et al., 1970) suggested that some protection from ruminal degradation without impairing intestinal release was achieved. However, Neudoerffer et al., (1971) reported that 30% breakdown occurred in the rumen and 60 - 65% of dietary methionine became available for post-ruminal absorption.

Grass and Unangst (1972) subsequently overcame the problem of poor release in the small intestine with a combination of tristearin and a liquid unsaturated fatty acid or oil. The Grass and Unangst preparation fed with a 14% CP diet to sheep increased nitrogen retention (Chalupa, 1975). Profiles of plasma methionine showed that methionine supplied by 10g of preparation (i.e. 2g methionine) was being utilized whereas methionine supplied by 20 and 30g supplements (i.e. 4 and 6g methionine) exceeded the animal's requirements. In studies where methionine was infused into the abomasum of sheep (Schelling et al., 1973; Chandler et al., 1972), 2 - 3 g/day maximised nitrogen retention, 4g/day was tolerable but 6g/day decreased nitrogen retention.

The encapsulation of methionine with formaldehyde-treated casein, gluten or gelatine was also investigated but

the solubility of methionine in aqueous solutions of these proteins made encapsulation difficult (Ferguson, 1975).

Other materials affording protection of amino acids include acyl esters (Ferguson, 1975), cellulose propionate-3-morpholino butyrate and imidamine polymers (Chalupa, 1975).

Methionine Hydroxy Analog: Extensive research has concerned the response of lactating cows to supplemental methionine added as methionine hydroxy analog (DL - α hydroxy γ - methyl mercapto butyrate calcium). Results have been positive for milk and fat production (Bishop, 1971; Griel et al., 1968; Bishop and Murphy, 1972; Chandler and Jahn, 1973; Polan et al., 1970a; Stanley and Toma, 1977), positive for the production of fat or fat test (Bouchard and Conrad, 1973a; Fosgate et al., 1973; Holter et al., 1972; Rosser et al., 1971; Van Horn et al., 1975; Chandler et al., 1976; Bhargava et al., 1977), or ineffective for either measure of production (Burgos and Olson, 1970; Fuquay et al., 1974; Hutjens and Schultz, 1971; Whiting et al., 1972; Olson and Grunbaugh, 1974; Wallenius and Whitchurch, 1975; Hutjens and Nold, 1975).

Holter et al., (1972) reported an increase in digestibilities of fiber and fat as a result of feeding methionine hydroxy analog (M-analog). Chandler et al., (1976) and Bhargava et al., (1977) did not find differences in efficiency of energy utilization for fat-corrected-milk production from M-analog-supplemented cows. Similarly, feed efficiency of calves did not improve when they were supplemented with M-analog (Gardner et al., 1972) or methionine (Ingalls et al.,

1970).

From the standpoint of basic nutrition, the magnitude of a response to supplemental methionine is interrelated strongly with other nutritional factors. The three most apparent factors are total dietary protein, sulfur and energy. The confounding of response to M-analog with that of elemental sulfur was discussed (Bull and Vandersall, 1973). Based on recently established sulfur requirements, (Bouchard and Conrad, 1973b,c,d) it seems that responses to methionine supplementation, when sulfur was at or above the requirement, would be due to the contribution of essential amino acids or to an effect of sulfur-containing amino acids in the rumen as noted from in vitro rumen fermentation studies (Gil et al., 1973c).

The response as a result of an essential amino acid would occur only under conditions where methionine was the first limiting amino acid (Nimrick et al., 1970a,b). These conditions are influenced strongly by the protein requirements as well as by the solubility and availability of proteins (Satter and Roffler, 1975; Burroughs et al., 1975). None of these responses would result unless energy was adequate.

The specific mechanisms which are responsible for increased fat production when M-analog is fed are not clear. Chandler et al., (1976) suggested that the response in fat probably is mediated through events in the rumen. Gil et al., (1973a,b, and c) established that M-analog and sulfur-containing amino acids stimulated rumen microbial growth