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
ad lib.	Ad Libitum
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
μM	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

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[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 (NH_3-N) content has been used as an index of proteolysis in grass silages. As silage DM decreased from 64.8 to 38.6%, NH_3-N (% total N) increased from 5 to 15% (Gordon et al., 1965). For a direct cut alfalfa silage (20% DM), the NH_3-N (% total N) level was 20.7% while a 53% DM wilted alfalfa haylage had an NH_3-N level of 9.5%. For corn plant material after ensiling, early harvested material (20% DM) had a tungistic acid soluble N level (% total N) of 52% while late cut material (50% DM) had a tungistic 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 NH_3-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 NH_3-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

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 anti-microbial 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 NH_3-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, NH_3-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 NH_3-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, live-weight 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 NH_3-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 NH_3-N release from corn silage water soluble N in vitro and in vivo respectively. Both studies indicated that when compared to urea, NH_3-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 NH_3-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 supression 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 insiduous 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 supression 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 Fennessy, 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 Fennessy (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 supression 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 summerized 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 artifical 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 B_1, a more slowly degradable fraction B_2 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 micro-organisms 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 biuratase 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. Anaerobosis 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 Elsden, 1960). This Y_{ATP} was initially estimated at 10g dry cells/ mole of ATP (Bouchop and Elsden, 1960). More recent research (Isaacson et al., 1975; Stouthamer and Bettenhaussen, 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 Bettenhaussen, 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 - diaminopimelic 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 Buttery (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 Hagemeister (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 micro-organisms) 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 artifically 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) (Georing 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 certian 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 Hagemeister (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 occured 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 Bharghava 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

beyond that possible as the result of inorganic sulfur or other amino acids. This stimulation brought about a more rapid utilization of glucose and ammonia and increased bacterial weight and nitrogen. In addition an increased digestibility of cellulose was established (Gil et al., 1973a; Bull and Vandersall, 1973; Polan et al., 1970b).

In a similar fashion, Patton et al., (1970) established that M-analog and methionine stimulated the growth of rumen protozoa in sheep. Protozoa are highly digestible and contain measurable lipid. This may be a partial explanation for the increase in fat production. In addition, lipid synthesis by micro-organisms in vitro has been increased markedly by methionine (Patton et al., 1968). The stimulation of rumen activity by M-analog was confirmed directly with in vivo experiments where increased dry matter and energy (Holter et al., 1972) and fiber (Bull and Vandersall, 1973; Holter et al., 1972; Polan et al., 1970b) digestion were observed and where methane production increased. Changes in ruminal volatile fatty acids, when analog is included in the diet, could offer an explanation for increased fat. Rosser et al., (1971) showed that molar proportions of ruminal butyrate increased and propionate decreased for cows fed M-analog as compared to controls. Another physiological alteration that may affect fat test is change in bovine fat metabolism beyond the rumen (Griel et al., 1968; McCarthy et al., 1968) when M-analog is included in the diet.

A direct postruminal metabolic action of M-analog has

not been established. Methionine was established as the first limiting amino acid for growing lambs on diets containing urea (Nimrick et al., 1970a,b). The feeding of encapsulated methionine to lambs in combination with certain protein supplements increased gain and feed efficiency (Mowat and Deelstra, 1972). In tissue metabolism studies with ruminants, M-analog had metabolic activity similar to that of methionine (Belasco, 1972; Reis, 1970). However, with lactating cows, methionine was not established always as the first limiting amino acid (Clark, 1975). By several experimental approaches with cows in varying stages of lactation, lysine, phenylalanine, histidine and threonine each have been the most limiting amino acid (Clark, 1975).

Even if methionine was the first limiting amino acid, some question exists as to dietary M-analog supplying methionine postruminally. Chalupa (1975) summarized literature supporting major degradation of M-analog in the rumen. The data of Bishop and Murphy (1972) and Belasco (1972) indicated that M-analog was more resistant to rumen degradation than methionine. With respect to the amount of the M-analog available postruminally, Papas et al., (1974) reported on the basis of blood plasma methionine levels, that the overall replacement value of M-analog for methionine was 23.6%. However, evidence has been obtained to support a methionine effect from dietary M-analog in that various catabolic compounds from methionine increased in blood as a result of M-analog feeding (Muller and Rodriguez, 1975; Polan and

Chandler, 1972). That the effect of M-analog is mediated via methionine was supported by the results of Remond et al., (1971) where 40 - 50g of DL methionine daily in the ration of lactating cows in early lactation increased milk fat production. Since the feeding of encapsulated methionine results in rumen bypass of methionine, the lack of response to this material (Broderick et al., 1970; Williams et al., 1970) supports the methionine effect being largely from the rumen.

The effect of M-analog on milk fat test in the absence of a response to dietary protein (Chandler et al., 1976) suggests that the action of M-analog in lactating cows is expressed in some fashion other than supplying the first limiting amino acid for protein synthesis by body tissues. This view is strengthened by the large number of observations (Bouchard and Conrad, 1973a; Fosgate et al., 1973; Holter et al., 1972; Rosser et al., 1971; Steele et al., 1973; Van Horn, 1975; Chandler et al., 1976), where milk fat production or fat test has been the only production parameter affected by M-analog supplementation.

Postruminal Administration of Proteins and Amino Acids

Protein nutrition in ruminants must be evaluated in terms of amino acids that are absorbed from the gut in relation to requirements for maintenance and production. It is believed that the dietary allowance of a lactating ruminant is sufficient when any further increase in the intake of

digestible crude protein fails to evoke a corresponding increase in milk production (Armstrong, 1968; Gordon and Forbes, 1970). The intake of digestible crude protein, therefore, bears little relationship to the actual quantities and proportions of amino acids presented for absorption (Satter and Roffler, 1975) and so it cannot be said with certainty that the amino acid supply from a particular intake of digestible crude protein is adequate in relation to the requirements for milk production.

Clark (1975) extensively reviewed results of lactational responses to postruminal administration of proteins and amino acids. Casein has been a source of protein in most postruminal infusion studies because it is the major milk protein and should offer an ideal pattern of amino acids for synthesis of milk protein (Clark, 1975).

Protein infusion: Postruminal infusion of casein has increased milk production 1 to 4 Kg/day, the greatest increase in milk yield being from the high producing cow (Clark, 1975). Cows producing less than 20 Kg/day seldom increased in milk yield more than 1 Kg/day (Hale et al., 1972; Vik-Mo et al., 1974a). Infusion of casein at 300 - 500g/day into the abomasum appears to produce maximum increases in milk yield (Clark, 1975). Generally, abomasal supplementation of 300 - 600g/day of casein had no effect on feed intake (Clark, 1975) or dry matter digestibility (Clark et al., 1977). However, Broderick et al., (1970) observed a significant depression in concentrate intake when 800g of casein supplemented with

methionine were infused into the abomasum.

Casein infused postruminally also significantly increases the crude protein percentage of milk and as a result of the milk yield response, a significant increase in milk crude protein yield of 10 to 15% is usual (Clark, 1975). Clark et al., (1977) obtained a 13.3% increase in milk protein production during infusion of sodium caseinate. Ranawana and Kellaway (1977b) observed a 20% increase in milk protein production in goats when 45g/day casein were abomasally infused. The lack of an increase in protein output in some trials was most probably due to low milk yield (Hale and Jacobson, 1972) and fluctuations in feed intake (Vik-Mo et al., 1974a). Vik-Mo et al., (1974a) indicated that the increase in crude protein yield of milk when casein was infused into the abomasum was due to an increase in both true protein and non-protein nitrogen of the milk.

In most trials, cows receiving abomasal infusions of casein produced milk with a slightly lower milk fat percentage than did control cows (Clark, 1975). This is due to an increased yield of milk and not to a depression in milk fat synthesis because milk fat yield was increased slightly by the casein supplementation (Broderick et al., 1970; Clark et al., 1973; Spires et al., 1973; Clark et al., 1977).

Nitrogen retention of lactating cows has been improved by infusing casein into the abomasum (Clark et al., 1977; Clark et al., 1973; Derrig et al., 1974). This would suggest that casein supplementation in the abomasum improves the

pattern of amino acids available for protein synthesis and thus, improves the efficiency of utilization of absorbed nitrogen. These data agree with the increased nitrogen balance in trials with steers (Chalupa et al., 1972), sheep (Colebrook and Reis, 1969) and goats (Ranawana and Kellaway, 1977a) when casein was infused into the abomasum. Clark (1975) suggested that the positive responses to postruminal casein infusions could be due to one or a combination of the following: (i) Infusion of casein increases the supply to the animal of one or more specific amino acids that are deficient in the digesta passing from the rumen. (ii) Casein infusions increase the supply of glucogenic amino acids to the liver and therefore increase the availability of glucose to the mammary gland. (iii) Casein infusions, directly or indirectly, effect the release of hormones likely to stimulate milk production.

Clark et al., (1977) reported an increase in arterial concentrations of most essential amino acids during infusion of sodium caseinate. These results were similar to those obtained from previous studies with ruminants in which the amino acid supply was increased by either increased dietary protein intake (Schelling et al., 1967) or postruminal protein infusion (Hogan et al., 1968; Spires et al., 1974; Derrig et al., 1974; Broderick et al., 1970).

Changes in plasma essential amino acid concentrations have been used to predict a limiting amino acid (Broderick et al., 1974). The concentration of all plasma free amino

acids do not increase at the same rate at which they are infused as sodium caseinate (Clark et al., 1977). However, when the supply of amino acids is increased by administering protein to a fed ruminant, it cannot be determined whether the plasma concentration of an amino acid changes as a result of its utilization for protein synthesis or its degradation.

Differences in the concentration of amino acids between arterial and mammary venous plasma can be employed to study the utilization of amino acids by the mammary gland. For meaningful information to be obtained from these measurements, it is necessary to express uptake of amino acids relative to their output in milk. Using this approach, Clark et al., (1977) reported that the estimated uptake of individual essential amino acids indicated that all were extracted by the mammary gland in sufficient quantities to provide for their output in milk of lactating cows. Similar studies with lactating goats (Ranawana and Kellaway, 1977a), indicated that methionine, threonine, phenylalanine and leucine were in short supply without infusion of casein.

An alternative approach for estimating the relative adequacy of individual amino acid supply to the mammary gland is to examine the total quantity of each amino acid available in plasma relative to total quantity of the amino acid secreted in milk. Data obtained by this method (Clark et al., 1977) suggested that the availability of either methionine, lysine or phenylalanine may be the most critical

supply for milk protein synthesis.

The second suggestion of Clark (1975) has been investigated by infusing similar quantities of casein and glucose postruminally in cows. In one experiment, milk production was increased by both casein and glucose (Vik-Mo et al., 1974a) while in others (Clark et al., 1973; 1977; Tyrell et al., 1972) milk yield was increased only by casein. Infusions of glucose postruminally into goats increased glucose entry rate but did not stimulate milk yield, while infusion of similar amounts of casein increased milk yield. With lactating cows (Clark et al., 1977), measurements of glucose entry rate showed a trend toward increased glucose flux when either glucose, sodium caseinate or glucose plus sodium caseinate were infused abomasally. The similarity in glucose entry rates obtained during infusion of glucose and sodium caseinate would suggest that the increase in milk production is not due totally to increased glucose flux resulting from sodium caseinate. Frobish and Davis (1977) showed that glucose infusion significantly ($P < .01$) increased milk yield but decreased ($P < .05$) milk protein % and fat test. Propionate infusion (15 moles/day) reduced feed intake but had no effect on milk yield and composition. Hartmann and Kronfeld (1973) indicated that it is not glucose availability per se that influences rate of milk secretion, but it is the quantity of glucose extracted by the mammary gland.

Ørskov et al., (1977) reported experiments in which glucose or casein were infused postruminally in early lactation

to see if mobilization of energy in support of lactation was limited by the amount of protein reaching the tissues or possibly by the availability of glucose for the synthesis of lactose in the mammary gland. Infusion of casein increased milk yield but caused a further decrease in the energy balance which was twice as great as with glucose infusion. The increase in negative energy balance was supported by the increase in the blood concentration of free fatty acids (Annison, 1960). Sparrow et al., (1973) observed the greatest milk yields and also the greatest weight loss with high yielding cows given a high-protein diet. The lack of response to infusion of glucose (Ørskov et al., 1977) suggests that the factor limiting milk yield was not glucose or glucose precursors for the synthesis of milk lactose, but protein for synthesis of milk protein. It is of course possible that with other basal diets glucose may be limiting yield.

Diet influences hormone secretion in ruminants (Trenkle, 1978). Cowie, (1966) and Machlin, (1973) reported that administration of growth hormone to lactating cows increases both milk yield and the efficiency of food utilization. Plasma growth hormone and insulin concentrations were increased in cattle, sheep and goats when amino acids either individually (arginine) or in mixtures (casein hydrolysate) were intravenously infused (Hertelendy et al., 1969; 1970; Stern et al., 1971; McAtee and Trenkle, 1971). Hart et al., (1975) reported that when compared to beef cows which tended to increase body tissue rather than produce milk, lactating cows had higher

circulating levels of growth hormone and lower levels of insulin and prolactin. Injecting synthetic thyrotropin releasing hormones, increased milk and milk protein yields (Convey et al., 1973). So it may be that responses in milk yield from abomasal supplementation of casein is elicited through hormone action.

Amino acid infusion: Abomasally infused casein consistently has increased production of milk and milk protein and nitrogen retention (Clark, 1975). Whether these responses are due solely to an increased supply of a single limiting amino acid or a group of equally limiting amino acids has not been clearly determined.

Indirect methods utilizing amino acid concentrations in plasma have been tried for identifying the limiting amino acid(s) for milk production and (or) milk protein synthesis in the lactating dairy cow. Chandler and Polan (1972) calculated minimum transfer efficiencies (transfer from blood to milk protein) and on this basis suggested that methionine, lysine, phenylalanine, tyrosine and threonine were most limiting. Using the same approach Vik-Mo et al., (1974b) reported that lysine and phenylalanine were the first two limiting essential amino acids when cows were infused abomasally with either casein or glucose. Derrig et al., (1974) suggested that threonine, phenylalanine and methionine were most limiting when casein was infused into the abomasum since these essential amino acids yielded the smallest increase in plasma relative to the amount infused in the form

of casein. Based on the relative uptake of each essential amino acid by the mammary gland with respect to output in milk, phenylalanine, histidine and methionine appeared most limiting when casein was administered postruminally (Derrig et al., 1974). In a similar study, Clark et al., (1977) suggested that the availability of either methionine, lysine or phenylalanine may be in the most critical supply for milk protein formation.

If a limiting essential amino acid will not accumulate in the plasma until its requirement is met (Mitchell et al., 1968; Stockland et al., 1970), then lysine, methionine and valine were most limiting when incremental amounts of formaldehyde-treated casein were added to a basal diet (Broderick et al., 1974).

Postruminal infusion of essential amino acids either singly or in combination has not given consistent positive response in lactating dairy cows (Schwab and Satter, 1973; 1974). This is in contrast to other data which show a significant increase in wool growth of sheep (Reis and Tunks, 1978; Reis, 1970) and nitrogen balance in steers (Oltjen et al., 1970; Chalupa et al., 1973). With the exception of a study by Fisher (1972), intravenous infusion of methionine has not stimulated production of milk, milk fat or milk protein (Fisher, 1969; Schwab et al., 1976; Teichman et al., 1969). Therefore it appears that methionine is probably not first limiting or that another amino acid is co-limiting with methionine for protein synthesis in the mammary gland.

Although dietary methionine hydroxy analog (MHA) supplementation has increased production of FCM (Bishop and Murphy, 1972; Griel et al., 1960; Holter et al., 1972) and milk yield (Bishop and Murphy, 1972), others (Burgos and Olson, 1970; Hutjens and Schultz, 1971; Whiting, 1972) reported no response from feeding MHA.

Schwab and Satter (1974) infused mixtures of essential amino acids into the abomasum and obtained increases in milk protein yield in lactating cows. Further studies (Schwab et al., 1976) showed that abomasal infusion of methionine had no effect on milk yield and composition. Lysine infusion resulted in 16% of the total response in yield of milk protein that was obtained with either the ten essential amino acids or sodium caseinate while infusion of lysine and methionine together accounted for 43% of the total response. This suggested that lysine and methionine were first and second limiting or co-limiting, for secretion of milk protein when rations based on corn, corn silage and alfalfa-grass hay were fed. In general, amino acid infusions had no effect on feed intake, milk fat and NPN content of milk (Clark, 1975).

Fababeans in Ruminant Rations

Fababeans (Vicia faba L.) have been introduced in Canada as a possible alternative to oilseed meals in diets for livestock. Being a legume, this crop has the advantage of fixing atmospheric nitrogen in the soil, thus benefiting the succeeding crop.

Improved varieties of Fababeans contain about 23 - 27% protein (Blair, 1978). A survey carried out in the U.K. by Eden (1968) indicated that the average protein content (DM basis) was 31.4% for spring beans (Minor's Tic) and 26.5% for winter beans (Throws MS), with lower protein values associated with large-seeded or early maturing varieties. The protein content of Canadian beans appears to be slightly higher than that of U.K. beans (Blair, 1978). Fababean protein is relatively high in lysine but low in methionine (Clarke, 1970). However, a high level of cystine partly overcomes the methionine deficiency.

The fat content is only 1.1 - 1.5%, which partly accounts for the low energy value. Fiber is about 6 - 9%. Pritchard et al., (1973) reported that winter beans contain 46 - 48% available carbohydrates (dextrins, starches and ethanol-soluble sugars) and 19 - 20% unavailable carbohydrates (lignin, cellulose, hemi-cellulose and water-soluble poly-saccaride). The corresponding values for spring beans were 30 - 42% and 22 - 37%.

Among undesirable constituents in Fababeans are condensed tannins (.34 - .5% DM) which markedly depress appetite and influence nutrient retentions in chicks (Marquardt et al., 1977) and decrease DM digestibility in vitro (Buckley, 1978). Another is a trypsin inhibitor, which can cause pancreatic enlargements in chickens (Wilson et al., 1972a). The trypsin inhibitor was detected in cotyledon and hull, the level in the hull being twice that in the cotyledon (Marquardt et al.,

1975). The activity of the inhibitor is destroyed by heating at 110° for 40 min. and is 1/5 the strength of an extract from raw SBM (Wilson et al., 1972b).

Hansen and Andersen (1972) fed dairy cows rations containing fodder beet, silage and a concentrate containing up to 60% Fababeans. Feed intake and milk yield were not affected. However, inclusion of beans in the concentrate led to higher fat and lower protein in the milk. Ingalls and McKirdy (1974) obtained satisfactory production performance of dairy cows by feeding concentrates containing 17.5% or 35% Fababeans. There was no significant effect on feed intake, milk production, milk protein or SNF content. The results also indicated that under fat-depressing conditions, the diet with the high level of Fababeans appeared to result in higher BF test compared with the diet containing rapeseed meal. Other data (unpublished) suggests no effect on butterfat test.

MacLeod et al., (1972) fed Holstein steer calves rations containing up to 30% beans. Total nitrogen retention with the bean rations was intermediate between that obtained with soy and fish meal rations. Dry matter digestibility of rations containing rolled barley and whole or rolled beans was similar at 80% when fed to 180 Kg steers. Ingalls et al., (1974) indicated that ground Fababean could replace soybean meal in calf rations. Levels of 24% in a starter and 30% in a grower ration were used to replace soybean meal and barley with no significant differences observed in feed intake, growth rate or feed efficiency during the periods from birth

to 7 weeks or from 7 to 20 weeks of age. Further studies with lambs (Ingalls et al., 1974) suggested that Fababeans would support similar rates of gain as other protein sources (rapeseed meal or SMB) but that feed efficiencies were poorer. Pelleting of the diet resulted in comparable performance as that obtained with SBM diets.

Smith and Sissons (1975) indicated that after preruminant calves have received a number of test feeds containing certain soybean products as the sole protein source they may develop abnormalities in digesta movement and nutrient uptake. The possibility of similar disorders developing in calves given feeds prepared from isolated field-bean (Vicia faba L.) protein was examined by Sissons and Smith (1975). Digesta flow and N digestibility did not differ markedly between feeds prepared from FB isolate and casein and there was no indication that FB isolate-feeds caused a development of digestive disorders anologous to those caused by feeds prepared from certain soybean products. Edwards et al., (1973) fed a pelleted ration containing 62% Fababean hulls and 38% of a concentrate mixture to sheep. The feed was consumed readily and there was no evidence of toxic or other deleterious effects. This work suggested that the hull fraction could be utilized successfully in ruminant feeding. Devlin et al., (1976) tested Fababean starch as a carrier for urea when fed to growing-finishing lambs. The results indicated that the starch/urea mixture supported almost as good growth in lambs as soybean meal and was better than a supplement of urea alone.

Zelter et al., (1970) studied the effect of formaldehyde treatment of horse beans (Vicia faba) on the solubility and susceptibility to deamination of the protein in vitro. Treatment reduced the solubility of the protein and its susceptibility to deamination. The best concentration of formaldehyde appeared to be 0.8% of the air dry seeds. Formaldehyde treatment had no effect on the biological value and true digestibility of the protein but it decreased available lysine. Pisulewski and Rys (1975) investigated the effect of formaldehyde treatment of horse beans on nitrogen balance, plasma urea levels, daily gains and conversion efficiency of concentrate diets in growing lambs. The treatment decreased the solubility of the horse bean protein, slightly reduced the rumen ammonia level but increased the plasma urea level. Nitrogen retention, daily gain and feed conversion efficiency were not affected by treatment.

Sharma and Nicholson (1975) reported no differences in DM intake, weight gain and feed efficiency among calves fed pelleted diets containing 8.4% SBM or 18% water or formaldehyde-treated FB; but formaldehyde treatment tended to improve the daily weight gain and feed efficiency of calves compared with diets containing SBM or water-treated FB. Formaldehyde treatment depressed the blood urea N and rumen fluid ammonia N levels of the calves. Apparent digestibilities of DM, CP and energy, as measured with mature sheep, were not different among the three diets. In a separate trial (Sharma and Nicholson, 1975) treatment of FB with formaldehyde or volatile

fatty acids (57% acetic acid and 39% propionic acid) tended to improve feed efficiency of growing calves and the flow of various nutrients through the gastrointestinal tract of fistulated sheep. These studies showed that FB protein is utilized well by growing dairy calves and mature sheep. Pisulewski and Rys (1975) studied the effect of formaldehyde treatment of horse beans on the extent of conversion of dietary protein into microbial protein and rumen ammonia-N in fistulated sheep. Formaldehyde treatment reduced the rumen ammonia-N level and the contribution of bacterial nitrogen to the total nitrogen in rumen digesta was lowered. However, treatment had no effect on the conversion of dietary protein into the rumen microbial protein.

Toynbee-Clarke (1970) investigated the potential of winter beans under whole-crop management and their value as a protein-rich supplementary feed. The results indicated that average DM yields of 106 hKg/ha can be obtained from whole crop beans with 13 to 18% CP. The data suggests prolonging the cutting date until the seeds within the pods are fully developed. Feeding experiments with beef cattle (Lonsdale and Tayler, 1969) have shown that maize silage supplemented with pelleted whole-crop beans produced a liveweight gain superior to that obtained from feeding maize silage ad lib. Supplementary feeding with bean pellets increased the daily DM intake as well as increasing the protein content of the feed.

McNight and MacLeod (1977) fed lactating Holstein cows

either whole plant FB silage or grass-legume silage as the sole roughage. Cows fed FB silage produced as much milk of similar protein and total solids contents as when grass-legume silage was fed. Milk from cows fed FB silage was higher in fat than that from grass-legume silage-fed cows although rumen VFA proportions were similar among the cows. Dry matter intake and body weight gain were greater for cattle fed FB silage, but apparent digestibilities of DM, protein and energy were not significantly different. The results suggest that the feeding value of FB silage for lactating dairy cows is comparable to that of good quality grass-legume silage.

PART ONE

Evaluation of Pro-Sil-treated Corn Silage and Fababeans in Dairy Rations

INTRODUCTION

The use of corn silage as a carrier of non-protein-nitrogen (NPN) in ruminant rations has become a world-wide practice. This method distributes NPN intake of cows over the entire day and masks the undesirable taste of urea (Huber, 1975). Milk yields of cows fed the NPN-treated silages have equalled or bettered those fed isonitrogenous rations from all natural protein (Knott et al., 1972; Huber and Thomas, 1971; Huber et al., 1973). Studies by Huber and Santana (1972) showed that aqueous ammonia was equal to urea as a NPN supplement in rations for growing heifers and lactating cows. Further studies (Huber et al., 1973) showed slightly higher milk persistencies for cows fed ammonia-treated silages compared to urea-treated silage. Compared to untreated silages, those treated with an ammonia-mineral suspension (Pro-Sil) are higher in lactic acid, water insoluble N and more stable when exposed to air (Huber, 1975; Soper and Owen, 1977; Britt and Huber, 1975). 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). The following experiments were designed to further evaluate Pro-Sil as a N source and to compare it to other N sources in dairy rations. The effect of adding encapsulated methionine to a high fababean dairy ration on milk yield and milk composition was also studied.

MATERIALS AND METHODS

EXPERIMENT 1

Whole corn plants (approximately 33% DM) were field chopped and treated with Pro-Sil (13.6% N) or urea (45% N). Pro-Sil was added, by use of a pump, into the suction side of the blower and the treated silage was blown directly into a concrete tower silo (4.9 x 15.2 m). The amount of Pro-Sil added was equivalent to 1 Kg/45 Kg of wet material (2.2%). Urea was distributed evenly on top of the load of silage in silage wagons just prior to delivery into the blower at a rate of 1 Kg/200 Kg of wet material (0.5%). The treated material was then blown directly into a wooden stave silo (4.3 x 14.0 m). Brome grass-alfalfa (50:50) sward was harvested at approximately 34 - 37% DM and was stored as direct cut grass-legume silage in a concrete tower silo (4.9 x 15.2 m). The silos were opened up for feeding after 3 months of storage.

Digestibility and Nitrogen Balance Trial with Sheep: Digestibility of the three silages (grass-legume, Pro-Sil-treated and urea-treated corn silages) was determined using 3 mature wether sheep (45 Kg body weight) in a 3 x 3 Latin square design. The silages were fed ad lib., twice daily. Sheep had access to clean water and 20g daily of a salt-mineral mixture. Each period consisted of 3 weeks preliminary and 1 week fecal collection. The sheep were transfered into metabolism crates three days prior to the collection period and were then fed

90% of their ad lib. consumption during the preliminary period. Plastic bags were fixed on the rump of each sheep with cement for separate collection of feces and urine. The bags were emptied several times daily and after weighing, the feces were stored in separate bags at -20°C. Urine was collected in plastic cans with added toluene to minimise N loss from the urine. Urinal samples were taken daily and stored at -20°C. Feed samples were taken daily and stored at -20°C. At the end of each period, feed, feces and urinal samples were composited for each animal, and subsampled for further analysis.

Feeding Trial with Dairy Cows: Eight Holstein cows in early lactation (4 to 8 weeks after parturition) were used to study the effect of NPN treatment of corn silage on milk yield and composition. A replicated 4 x 4 Latin square changeover design was employed. Each experimental period consisted of 2 weeks for adjustment and 2 weeks for comparison. Cows received grass-legume, Pro-Sil-treated corn or urea-treated corn silage ad lib. plus 2 Kg of hay (alfalfa-brome grass) per day. A concentrate mixture (Table 1) was fed twice daily to the individual cow to meet her energy requirements for milk produced over 9 Kg/day for a medium level and over 16 Kg/day for a low level of grain feeding regime. The experimental treatments intended to be isonitrogenous were as follows:

1) Grass-legume plus concentrate mixture A.
2) Urea-treated corn silage plus concentrate mixture B.
3) Pro-Sil-treated corn silage plus concentrate mixture B.

4) Pro-Sil-treated corn silage plus concentrate mixture C. Treatments 1, 2 and 3 were with the medium level of grain feeding while treatment 4 was with the low level of grain feeding.

The cows were housed in a stanchion barn and were fed individually. Feed and orts were weighed daily and recorded. Cows had direct access to water through automatic watering bowls. Wood shavings were used for bedding. The cows were weighed at the beginning and end of each period.

The cows were milked twice daily and production was recorded. Two 24h-period milk samples were taken weekly for milk composition (fat, protein and solids-not-fat) determination. Silage and grain mixtures were sampled weekly and were composited for each period.

Digestibility Trial: During the last week of each period, total collection of feces and urine was carried out on 4 animals in one square for 3 days. Urine was collected by use of urinary catheters into plastic containers that contained toluene and was measured and sampled daily. Daily aliquots of urine were stored at -20°C and composited by volume at the end of each collection period. Total fecal excretion of each cow was collected twice daily and daily samples were taken and composited at the end of the 3-day period. Feed and orts were sampled daily and then pooled for each period. On the last day of each collection period, rumen fluid samples were taken via a stomach tube 2½h after the morning feeding. At the same time blood samples were collected from the caudal vein for plasma urea-N analysis.

Table 1. Ingredient and Chemical Composition of Concentrate Mixtures and Hay* Fed to Lactating Dairy Cows (Expt. I)

	Concentrate Mixtures		
	A	B	C
Ingredients	——% air dry basis ——		
Rolled barley	83.5	85.9	81.3
Soybean meal	10.5	6.6	10.7
Cane molasses	3.0	3.0	3.0
Calcium phosphate	2.0	2.0	2.0
Trace mineral salt	0.5	0.5	0.5
Limestone	-	1.5	2.0
Vitamin premix[1]	0.5	0.5	0.5
Chemical Composition (%DM)			
Crude protein	18.7	16.2	17.4
Acid-detergent fibre	9.2	8.7	9.3
Gross energy (Kcal/g)	4.36	4.30	4.43
0.15 M NaCl-Soluble N(% total N)	12.1	12.9	11.7

*Hay contained (%DM) 17.8 CP, 42.0 ADF, 4.60 Kcal/g (GE) and 25.0% soluble N (% total N).

[1]2.27 Kg premix contained 2,000,000 IU Vit. A, 200,000 IU Vit. D, 5,000 IU Vit. E, 908g MgO, 454g Sulfur, 114g ZNO, 21g $MnSO_4.H_2O$ per 454 Kg mixed feed.

EXPERIMENT II

Whole corn plants (approximately 38 - 42% DM) were field chopped and stored as untreated or Pro-Sil treated silage. Pro-Sil (13.6% N) was added at a rate of 1 Kg/75 Kg of wet material (1.3%) and the silage was stored in a concrete tower silo (4.9 x 15.2 m). The untreated material was stored in a wooden stave silo (4.3 x 14.0 m). Whole corn plants (approximately 32% DM) from another field were field chopped and stored alternately in two adjacent concrete bunker silos (5.2 x 14.6 x 2.7 m) as Pro-Sil or urea-treated silage. Pro-Sil was added at a rate of 1 Kg/60 Kg of wet material (1.7%) while urea (45% N) was added at a rate of 1 Kg/200 Kg of wet material (0.5%). The bunker silos were covered with a plastic sheet and bales of hay to exclude air and water. The silos were opened up for feeding 2 to 3 months after storage.

Digestibility and Nitrogen Balance Trial with Sheep: Digestibility of the 4 silages (Pro-Sil treated, 1.3% and 1.7%, urea-treated and untreated corn silages) were determined with 4 wether sheep (50 Kg body weight) in a 4 x 4 Latin square design. Each period consisted of 3 weeks preliminary and 1 week collection. The silages were fed ad lib. twice daily. Sheep had access to clean water and 20g of a salt-mineral mixture. Three days before the collection period, all sheep were fed 90% of their ad lib. consumption during the preliminary period. Plastic bags were attached to the rump of each sheep for separate collection of feces and urine. The bags were emptied several times daily and the feces were stored in

separate plastic bags for each sheep at -20°C after recording their weight. Urine was collected in metal trays with added toluene placed underneath the slatted floor area of each stall. Daily aliquots of urine were stored at -20°C and were then composited at the end of each period. Silage samples were taken daily during each collection period. At the end of each collection period, silage and fecal samples were composited for each animal and subsampled for further analysis.

Feeding trial with dairy cows: Eight Holstein cows in early lactation (4 to 10 weeks after parturition) were used to compare fababeans with Pro-Sil-treated corn silage as sources of protein for lactating dairy cows and to study the effect of adding encapsulated methionine to a high fababean grain ration on milk yield and composition. A replicated 4 x 4 Latin square changeover design was employed. Cows received Pro-Sil-treated (1.3%) or untreated corn silage and a concentrate mixture (Table 2) in a 45:55 (DM basis) as a complete feed plus 2 Kg of alfalfa-brome grass hay. The experimental treatments intended to be isonitrogenous were as follows:

1) Pro-Sil-treated corn silage plus grain mixture P (Pro-Sil diet).
2) Untreated corn silage plus grain mixture U (Urea diet).
3) Untreated corn silage plus grain mixture M (Fababean plus methionine diet).
4) Untreated corn silage plus grain mixture F (Fababean diet).

Table 2. Ingredient and Chemical Composition of Concentrate Mixtures and Hay* fed to Lactating Dairy Cows (Expt. II)

	Concentrate Mixtures			
	SBM (P)	SBM+UREA (U)	Fababeans+MET (M)	Fababeans (F)
Ingredients	% air dry basis			
Rolled barley	71.9	63.9	37.1	35.7
Soybean meal	6.4	12.5	-	-
Fababean grain	-	-	40.0	42.0
Encapsulated methionine[1]	-	-	0.6	-
Urea	-	1.3	-	-
Oats	10.0	10.0	10.0	10.0
Tallow	5.0	5.0	5.0	5.0
Cane molasses	3.0	3.0	3.0	3.0
Limestone	2.0	2.5	2.5	2.5
Calcium phosphate	0.7	0.8	0.8	0.8
Trace mineral salt	0.5	0.5	0.5	0.5
Vitamin premix[2]	0.5	0.5	0.5	0.5

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Table 2. CONTINUED	Concentrate Mixtures			
	SBM (P)	SBM+UREA (U)	Fababeans+MET (M)	Fababeans (F)
Chemical Composition (%DM)				
Crude protein	13.4	20.8	17.2	17.2
Acid-detergent fibre	9.3	8.8	9.8	10.7
Gross energy (Kcal/g)	4.57	4.55	4.53	4.55
Calcium	1.16	1.49	1.51	1.43
Phosphorus	0.52	0.60	0.59	0.59
0.15 M NaCl-Soluble N(% total N)	14.6	26.9	15.3	14.3

*Hay contained (%DM) 17.0 CP, 35.7 ADF, 4.45 Kcal/g (GE), 0.72 Calcium, 0.18 Phosphorus and 25.1% Soluble N (% total N).

[1]Encapsulate methionine contained 20% methionine, supplied by Delmar Chemicals Co. Canada.

[2]2.27 Kg of premix contained 2,000,000 IU Vit. A, 200,000 IU Vit. D, 5,000 IU Vit. E, 908g MgO, 114g ZnO, 15g $MnSO_4.H_2O$ per 454 Kg mixed feed. In addition premix for grain U contained 454g Sulfur.

Each experimental period consisted of 2 weeks of adjustment and 2 weeks of comparison. The cows were housed and managed in a similar manner to those in experiment I. Feed intake data, milk data and samples, digestibility trial data and rumen fluid samples were collected following the same procedures outlined in experiment I. Blood samples were collected from the caudal vein for 3 consecutive days, 2½h after the morning feeding during the last week of each period for blood plasma urea-N and amino acid analyses.

Laboratory Procedures: The same methods and procedures of laboratory analyses were used for experiments I and II. Milk samples were analyzed for butterfat with Milko-tester (AOAC, 1975), protein by the acid orange G dye binding method (Ashworth et al., 1960) and solids-not-fat by the plastic beads of Golding (1959).

Dry matter in feeds, orts and feces was determined by drying at 60°C to a constant weight in a forced air oven. Nitrogen was determined on wet silage samples, dried feed samples, fecal samples and urine by the macro-Kjeldahl method (AOAC, 1970). Gross energy was determined on dried samples with an adiabatic oxygen bomb calorimeter. Acid-detergent-fibre was determined according to Goering and Van Soest (1970).

For chemical analysis of silage, 20g of fresh silage were added to 180 ml of distilled water and homogenized in a Sorvall Omni Mixer at full speed for 3 minutes. This was called the 'homogenate'. After filtering the 'homogenate' through 2 layers of cheesecloth, one portion was centrifuged at 15,000

RPM for 10 minutes. The resulting supernatant was used directly for WSN determination or was treated with 10% (w/v) tricholoroacetate for non-protein-nitrogen determination by the micro-Kjeldahl procedure (AOAC, 1970). The remaining filtered 'homogenate' was treated with 50% (w/v) sulfosalicylic acid (SSA) to bring the final concentration to 5% SSA. The mixture was centrifuged at 15,000 RPM for 10 minutes. The supernatent (SSA extract) was used for VFA and lactic acid determination. A glass electrode pH meter was used to determine the pH of the 'homogenate'. Lactic acid determination was made on the SSA extract using the method described by Barker and Summerson (1941).

Volatile fatty acids in rumen fluid and silage samples were determined by the method of Erwin et al., (1961). One ml of 25% (w/v) metaphosphoric acid was mixed with 5 ml of strained rumen fluid or the SSA extract, allowed to stand for thrity minutes and centrifuged at 1500 x G for 10 minutes. The supernatant was analyzed for VFA by gas liquid chromatograph with a hydrogen flame ionization detector. The apparatus employed a 180 x 0.318 cm O.D. stainless steel column packed with 20% Neopentyl succinate and 2% H_3PO_4 on 60/80 mesh Gas Chrom R and conditioned 17h at 205°C. Helium (30 psig) was used as a carrier gas with a flow rate of 50 ml/min. plus hydrogen (26 psig) with a flow rate of 50 ml/min. and air (20 psig) with a flow rate of 330 ml/min. The gas liquid chromatograph was attached to a Honeywell recorder. Ammonia-N in rumen fluid was determined using an ammonia

electrode (Orion Model 95 - 10). Blood plasma urea-N was analyzed with an autoanalyzer (Marsh et al., 1965).

Soluble nitrogren was determined on dried feeds in a 0.15M NaCl solution (Crooker et al., 1978). The amount of feed containing 50 mg N was added to 200 ml solvent. After adjusting the pH to 6.5, the mixture was incubated at 39°C while being constantly stirred for 1h. The homogenate was filtered through Whatman no. 4 filter paper in a buchner funnel. Fifty ml aliquot of the filtrate was taken for N determination by the micro-Kjeldahl method (AOAC, 1970).

Amino acid analysis of diets in Experiment II were carried out according to the procedures outlined by Bragg et al., (1966) with modifications as described by Giovannetti et al., (1970) on a 116-Beckman model amino acid analyzer. For normal hydrolysis 50 mg of the sample combined with 6N HCl were autoclaved for 16h at 121°C, the tubes being evacuated. For methionine and cystine analysis the method described by Hirs (1967) was used. Two ml performic acid were added to 50 mg of the sample which was then placed on ice for 20h to form a homogenous mixture. Hydrobromic acid (0.3 ml) was then added to destroy the excess reagent. The sample was then evaporated to dryness and the tube containing the sample was evacuated by autoclaving for 16h at 121°C.

Plasma samples were deproteinized according to the procedures of Folin and Wu, (1919). To 2 ml of blood plasma, 1 ml of 0.6N H_2SO_4 and 1 ml of 10% (w/v) sodium tungstate

solution were added. The precipitate was removed by centrifuging at 2500 RPM for 20 min. The supernatent was analyzed for amino acids according to the method of Spackman et al., (1958). Analysis was performed on the long column using lithium citrate buffers to obtain all amino acids up to and including tyrosine.

Statistical Analysis: The data collected in experiments I and II were analyzed statistically as a Latin square design and the treatment means were subjected to the Students-Newman-Keul (SNK) test (Snedecor and Cochran, 1967). No statistical analysis was made on the amino acid composition of the experimental diets (Table 3).

Table 3. Amino Acids Composition of Diets Fed to Lactating Dairy Cows (Expt. II)*

Amino Acid	Corn Silages		Concentrate Mixtures				Hay
	Untreated	Pro-Sil treated	P	U	M	F	
	g/100g total aa						
Lysine	3.55	3.06	4.70	4.57	4.64	5.61	5.54
Histidine	1.81	1.73	2.30	1.91	2.45	2.42	2.14
Arginine	2.75	2.57	5.61	6.40	8.05	7.97	4.64
Aspartic acid	10.23	11.54	10.21	11.12	11.91	12.15	14.32
Threonine	4.59	3.75	3.94	4.12	3.93	3.97	5.18
Serine	4.25	3.86	4.28	4.43	4.53	4.57	5.71
Glutamic acid	17.10	16.12	23.33	22.42	21.05	21.49	12.44
Proline	8.39	7.62	7.63	7.44	5.76	5.84	7.46
Glycine	4.65	4.05	4.57	4.46	4.59	4.57	5.48
Alanine	10.61	16.74	4.82	4.65	4.59	4.45	6.33
Cystine	0.99	1.60	1.62	1.98	1.44	1.41	1.42
Valine	6.26	5.45	5.71	5.54	5.70	5.61	6.71

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Table 3. CONTINUED

Amino Acid	Corn Silages		Concentrate Mixtures				Hay
	Untreated	Pro-Sil treated	P	U	M	F	
Methionine[1]	1.05	1.46	2.00	1.60	1.67	0.91	1.66
Isoleucine	4.22	3.56	4.49	4.54	4.27	4.59	5.10
Leucine	11.19	9.93	7.81	7.74	7.80	8.00	8.67
Tyrosine	1.71	1.46	1.36	1.93	1.79	1.66	1.80
Phenylalanine	6.65	5.53	5.61	5.16	4.81	4.77	6.48

*All values are single analysis of composited samples, and were not statistically analyzed.

[1]Calculated value for concentrate mixture M was 1.63.

RESULTS

EXPERIMENT 1

Silage Chemical Composition: Application of Pro-Sil appeared to result in less N content of the silage and less N recovery compared to urea treatment (Table 4). The proportion of total N that was in water-soluble form was slightly higher in the NPN-treated corn silages compared to the grass-legume silage. Urea-treated corn silage N appeared to be more soluble in 0.15M NaCl solution than the other two silages. Pro-Sil treatment appeared to result in higher total organic acid, lactic acid and acetic acid contents than urea treatment. The grass-legume silage had lower levels of lactic acid and higher pH than the NPN-treated silages.

Digestibility and Nitrogen balance trial with sheep: Silage DM consumption was not different ($P > .05$) among treatments (Table 5). Silage DM intake (% body weight or $g/KgW^{3/4}$) was somewhat similar for the NPN-treated silages but slightly higher ($P > .05$) for the grass-legume silage. Sheep fed the Pro-Sil-treated corn silage gained slightly less ($P > .05$) weight than those fed either urea-treated corn silage or grass-legume silage.

Apparent digestibilities of DM, CP and energy were higher ($P < .05$) for the NPN-treated corn silages compared to the grass-legume silage. Non-protein-nitrogen source appeared to have no effect on the apparent digestibilities of DM, CP or energy.

Table 4. Chemical Composition of Experimental Silages (Expt. I)

Items	Silages		
	Grass-legume	Urea-treated corn	Pro-Sil treated corn
Dry matter content, %	38.5	31.0	31.5
	% DM		
Crude protein	12.94	12.62	12.25
Acid-detergent fiber	44.39	30.97	31.30
Gross energy (Kcal/g)	4.42	4.37	4.32
Total organic acids	6.03	6.61	8.95
Lactic acid	2.38	3.87	5.38
Acetic acid	2.86	2.52	3.15
Butyric acid	0.64	0.22	0.38
Propionic acid	0.15	-	0.04
pH	4.45	3.75	4.00
% total N			
Water-soluble N	60.4	64.4	62.8
Water-soluble NPN	36.0	41.2	44.9
0.15M NaCl-Soluble N	51.2	59.0	52.7
Ammonia-N	3.0	2.1	5.6
Recovery of added N[1] (%)	-	100	95

[1]Based on the N content of the material ensiled and the N content of the material removed from the silo.

Table 5. Average Daily Dry Matter Consumption and Apparent Digestibility Coefficients by Sheep Fed Experimental Silages (Expt. I)

Items	Silages: Grass-legume	Silages: Urea-treated corn	Silages: Pro-Sil treated corn	SE[1] ±
Dry Matter intake				
g/day	1017	997	1000	11
% body weight	2.35	2.26	2.20	0.06
$g/KgW^{3/4}$	60.3	58.1	57.1	4.0
Body weight gain (g/day)	214	190	43	26
Apparent digestibility coefficients %				
Dry matter	57.3[a]	65.8[b]	67.0[b]	1.0
Crude protein	64.7[a]	69.4[b]	68.7[b]	0.2
Gross energy	56.2[a]	66.4[b]	68.3[b]	1.1

[1]Standard error of the mean.

[a,b]Means with different superscripts are significantly different ($P<.05$).

Average daily N intake was not different ($P > .05$) among treatments (Table 6). The proportion of N consumed that was excreted in feces was higher ($P < .05$) for sheep fed the grass-legume silage than for those fed the corn silages. Sheep fed the Pro-Sil-treated corn silage excreted less ($P < .05$) N in urine than those fed either grass-legume or urea-treated corn silage. Nitrogen consumed that was retained was not different ($P > .05$) among treatments, but was slightly lower for sheep fed the grass-legume silage compared to sheep fed the corn silages. Nitrogen retained as a percentage of N absorbed was not different ($P > .05$) among treatments but was lowest for sheep fed the grass-legume silage. Pro-Sil-treated corn silage resulted in slightly better N utilization than urea-treated corn silage though the difference was not significant ($P > .05$).

Rumen ammonia-N (RAN) and blood plasma urea-N (BUN) concentrations were not different ($P > .05$) among treatments (Table 7). Total ruminal volatile fatty acid (VFA) concentration was not different ($P > .05$) among treatments. The molar percentage of acetic acid was lower ($P < .05$) for sheep fed the Pro-Sil treated corn silage compared to those fed either of the other two silages. The molar percentage of butyrate tended to be lower ($P < .10$) for sheep fed the grass-legume compared to sheep fed the Pro-Sil treated corn silage. The acetate to propionate ratio was not different ($P > .05$) among treatments but was lowest for sheep fed the Pro-Sil-treated corn silage.

Table 6. Nitrogen Utilization By Sheep Fed Experimental Silages (Expt. I)

Items	Silages			
	Grass-legume	Urea-treated corn	Pro-Sil treated corn	SE±
Nitrogen balance, g/day				
Ingested	20.7	21.4	19.2	1.1
Fecal	7.3	6.5	6.0	0.4
Urine	10.4[a]	10.4[a]	7.9[b]	0.7
Total excreted	17.7	16.9	13.9	1.1
Absorbed (apparent)	13.4	14.9	13.2	0.8
Retained	3.0	4.5	5.3	1.2
Percent of intake				
Fecal	35.3[a]	30.6[b]	31.3[b]	0.2
Urine	50.3	48.0	42.4	4.8
Absorbed (apparent)	64.7[a]	69.4[b]	68.7[b]	0.2
Retained	14.4	21.4	26.3	4.6
Percent of apparently absorbed N retained	22.3	30.9	38.4	6.8

[a,b]Means with different superscripts are significantly different ($P < .05$).

Table 7. Rumen Volatile Fatty Acid Concentration and Molar Ratios, Ammonia-N and Blood Plasma Urea-N Levels of Sheep Fed Experimental Silages (Expt. I)

Items	Silages			
	Grass-legume	Urea-treated corn	Pro-Sil treated corn	SE±
Rumen ammonia-N (mg/100ml)	14.4	14.0	9.9	1.3
Plasma urea-N (mg/100ml)	14.5	16.2	16.0	1.0
Total VFA, mM/l	94.3	88.6	73.9	3.6
Individual VFA (molar %)				
Acetic	66.57[a]	59.73[a]	48.91[b]	1.46
Propionic	21.08	21.82	22.03	1.31
Butyric	8.08[c]	15.12[cd]	26.57[d]	2.73
Isobutyric	0.99	1.00	0.97	0.18
Valeric	1.95	1.15	0.84	0.51
Isovaleric	1.33	1.18	0.68	0.08
Acetate:propionate	3.16	2.74	2.22	0.20

[a,b]Means with different superscripts are significantly different ($P < .05$).

[c,d]Means with different superscripts are significantly different ($P < .10$).

Feed Consumption by Dairy Cows: Silage DM consumption (Table 8) was not different ($P > .05$) among the treatments, but there was a slight increase in Pro-Sil-treated corn silage intake with the low level of grain feeding. Concentrate DM consumption was significantly ($P < .05$) decreased with the low level compared to the medium level of grain feeding. Total DM consumption was lower ($P < .05$) for the low grain-fed cows than those fed either urea-treated corn or grass-legume silage-containing diets. There was no significant ($P > .05$) difference between silage DM consumption within NPN sources. Total feed consumed per unit body weight was not different ($P > .05$) with the medium level of grain feeding but declined significantly ($P < .05$) with the low level of grain feeding. The forage to concentrate ratio was approximately 2:1 for the medium level and 3:1 for the low level of grain feeding.

Total N consumption of cows fed the Pro-Sil-treated corn silage-low grain diet was lower ($P > .01$) than those fed the grass-legume silage diet. Cows fed either the grass-legume or urea-treated corn silage diets consumed more ($P < .05$) N than those fed the Pro-Sil-treated corn silage diets. Intake of soluble N was not different ($P > .05$) among treatments. Insoluble N intake was higher ($P < .05$) for cows fed the grass-legume silage diet compared to those fed the Pro-Sil treated corn silage-low grain diet. Soluble N (% of N intake) was higher ($P < .05$) for the urea-treated corn silage diet compared to the grass-legume silage diet.

Table 8. Effect of Silage type and Grain Level on Dry Matter Consumption of Dairy Cows (Expt. I)

	Diets				
Silage Grain Level Items	Grass-legume Medium	Urea-corn Medium	Pro-Sil-corn Medium	Pro-Sil-corn Low	SE±
Dry matter intake (Kg/day)					
Silage	12.3	11.8	11.4	12.1	0.5
Concentrate	6.6^{a}	6.4^{a}	6.2^{a}	4.2^{b}	0.5
Hay	1.8	1.8	1.8	1.8	-
Total	20.7^{a}	20.0^{a}	19.4^{ab}	18.1^{b}	0.5
Total feed (% body weight)	3.33^{a}	3.26^{a}	3.14^{a}	2.89^{b}	0.07
Silage (% total feed)	59.4	59.0	58.8	66.9	-
Forage:concentrate	68.32	68:32	68:32	77:23	-
Composition of total ration					
Acid detergent fibre, %	32.9	24.8	25.0	27.2	-
Gross energy (Mcal/Kg DMI)	4.42	4.37	4.32	4.35	-
Crude protein, %	15.2	14.2	14.0	14.0	-
Total N intake, g/day	503^{Aa}	455.0^{ABb}	435^{ABb}	405^{Bb}	15
Soluble N	167	175	151	151	8
Insoluble N	336^{a}	280^{b}	284^{b}	254^{b}	15
Soluble N (% of intake)	33.1^{a}	38.4^{b}	34.8^{ab}	37.4^{ab}	1.3

^{A,B}Means with different superscripts are significantly different ($P < .01$).

^{a,b}Means with different superscripts are significantly different ($P < .05$).

Milk Yields and Composition: The mean daily milk and FCM yields were not different ($P > .05$) among treatments (Table 9). Milk content of fat, protein, solids-not-fat and total solids were not different ($P > .05$) among treatments. Reducing the amount of concentrate fed did not affect daily milk or FCM production at the level of production found in this experiment. Milk fat and protein yields were not affected by either forage source or grain level. Mean milk yield as a percentage of pre-experimental milk yield (persistency) was not different ($P > .05$) among treatments.

Digestibility and N Balance Trial: Apparent digestibilities of DM and energy were lower ($P < .05$) for cows fed the grass-legume diet than for those fed the corn silage diets (Table 10). Apparent digestibilities of CP and ADF were not different ($P > .05$) among treatments; however the apparent digestibility of ADF was slightly lower for the diet containing grass-legume silage compared to the diets containing corn silage. Average daily intake of digestible CP, ADF and energy were not different ($P > .05$) among treatments. Cows fed the low level of grain consumed slightly less ($P > .05$) digestible CP and energy compared to cows fed the corn silage-medium grain diets.

Average daily N intake was not different ($P > .05$) among treatments, but was highest for cows fed the grass-legume diet and lowest for cows fed the low grain level (Table 11). Cows receiving the low grain level also consumed less ($P < .05$) insoluble N compared to those fed the grass-legume diet.

Table 9. Effect of Silage Type and Grain Level on Milk Yields and Milk Composition of Dairy Cows (Expt. I)

	Diets				
Silage / Grain Level / Items	Grass-legume Medium	Urea-corn Medium	Pro-Sil-corn Medium	Pro-Sil-corn Low	SE±
Milk yield (Kg/day)	22.1	23.1	22.3	22.1	0.4
Butterfat, %	4.00	3.88	3.92	4.02	0.08
4% Fat-corrected milk (Kg/day)	22.1	22.7	22.0	22.2	0.4
Protein, %	3.62	3.70	3.70	3.64	0.04
Solids-not-fat, %	8.84	8.90	8.85	8.92	0.04
Total Solids, %	12.84	12.78	12.77	12.94	0.12
Butterfat yield (Kg/day)	0.88	0.89	0.87	0.88	0.02
Protein yield (Kg/day)	0.79	0.84	0.82	0.80	0.02
Milk persistency[a], %	76.6	79.9	77.4	76.1	1.3

[a]Milk persistency = 100 x (Treatment milk yield/Pre-experimental milk yield).

Table 10. Apparent Digestibility Coefficients and Digestible Nutrient Intake of Cows Fed Experimental Diets (Expt. I)

Items	Diets: Grass-legume Medium	Urea-corn Medium	Pro-Sil-corn Medium	Pro-Sil-corn Low	SE±
Silage / Grain Level					
Apparent digestibility coefficients, %					
Dry matter	60.4[a]	67.5[b]	68.8[b]	66.4[b]	1.7
Crude protein	64.5	66.4	64.3	65.2	1.7
Acid-detergent fibre	43.3	50.5	49.9	50.4	2.5
Gross energy	59.0[a]	67.8[b]	68.0[b]	66.0[b]	1.5
Digestible nutrient intake					
Crude protein (Kg/day)	1.88	1.85	1.62	1.51	0.13
Acid-detergent fibre (Kg/day)	2.95	2.50	2.43	2.48	0.12
Energy (Mcal/Kg/DMI/day)	2.61	2.96	2.94	2.89	0.20
CP intake/NRC requirements[1] (%)	129.5	114.3	112.4	104.5	-
DE intake/NRC requirements (%)	105.5	113.4	111.5	102.1	-

[a,b]Means with different superscripts are significantly different ($P < .05$).

[1]Protein and DE requirements for maintenance plus milk production estimated from NRC (1978) Tables.

Fecal and urinal N losses were not different ($P > .05$) among treatments. The mean quantity of N apparently absorbed and N excreted in milk were not different ($P > .05$) among the treatments. Average daily N retained was highest for cows fed the grass-legume diet, medium for cows fed the corn silage-medium grain diets and lowest for cows fed the corn silage-low grain diet, although the differences were not significant ($P > .05$). Productive N (N retained + N in milk) followed the same trend as N retained.

The percentage of dietary N excreted in feces and urine were not different ($P > .05$) among treatments, but the percentage of dietary N recovered in milk was lower ($P > .05$) for cows fed the grass-legume diet than for those fed the Pro-Sil-treated corn silage diets. Non-protein-nitrogen source and grain level did not affect the percentage of dietary N recovered in milk. Nitrogen retained as a percentage of N intake was similar with the medium level of grain feeding but decreased ($P < .05$) with the low level of grain feeding. The efficiency of conversion of apparently absorbed N into milk N was slightly higher for cows fed the corn silage diets but not different ($P > .05$) from those fed the grass-legume silage diet. Cows fed the grass-legume silage diet retained slightly more N absorbed than those fed the corn silage diets, but the difference was not significant ($P > .05$). Among the corn silage diets, cows fed the low level of grain excreted almost all the N apparently absorbed into urine and milk. The proportion of N intake or N absorbed that was productive N

Table 11. Nitrogen Intake and Utilization and Body Weight Changes of Cows Fed Experimental Diets (Expt. I)

	Diets				
Silage	Grass-legume	Urea-corn	Pro-Sil-corn	Pro-Sil-corn	
Grain Level	Medium	Medium	Medium	Low	
Items					SE±
Nitrogen balance, g/day					
Ingested	463	441	406	370	27
Soluble N	161	182	146	148	14
Insoluble N	302[a]	259[ab]	260[ab]	222[b]	15
Fecal	163	148	146	128	11
Urine	126	136	106	123	13
Milk	114	123	121	113	3
Absorbed (apparent)	300	293	260	242	20
Retained[1]	60	34	33	6	19
Productive[2]	174	157	154	119	20
Percent of N intake					
Soluble N	34.6[a]	41.2[b]	35.9[a]	40.0[b]	1.1
Fecal	35.5	33.6	35.7	34.8	1.7
Urine	27.2	30.8	26.4	34.2	3.3
Milk	24.8[a]	28.0[ab]	29.7[b]	30.4[b]	1.1
Absorbed (apparent)	64.5	66.4	64.3	65.2	1.2
Retained	12.5	8.4	8.2	1.6	4.5
Productive	37.2	36.4	37.5	32.0	3.8

CONTINUED....

Table 11. CONTINUED

Items	Diets: Grass-legume Medium	Urea-corn Medium	Pro-Sil-corn Medium	Pro-Sil-corn Low	SE±
Silage					
Grain Level					
Percent of apparently absorbed N					
Urine	42.3	46.4	42.2	53.1	5.2
Milk	38.6	42.0	46.5	46.8	2.3
Retained	19.1	11.6	11.3	2.4	5.6
Productive	57.7	53.6	57.8	49.2	5.0
Body weight gain, Kg/day	0.11	0.15	0.16	-0.22	0.17

[1]N retained = N intake - (Fecal N + urinal N + Milk N).

[2]Productive N = Milk N + Retained N.

[a,b]Means with different superscripts are significantly different ($P < .05$).

was not different ($P > .05$) among treatments. All cows gained weight during the experiment, except those fed the low level of grain but this difference was not significant ($P > .05$) (Table 11).

Rumen fluid VFA and ammonia-N and plasma urea-N levels: Rumen fluid RAN content was not different ($P > .05$) among treatments (Table 12). Blood plasma urea-N concentration was higher ($P < .05$) for cows fed either the grass-legume or urea-treated corn silage diet than for those fed the Pro-Sil-treated corn silage diets.

Total ruminal VFA concentration was not significantly ($P > .05$) affected by treatment, nor was the molar percentage of isobutyrate (Table 12). The molar percentage of acetate was significantly ($P < .01$) higher for cows fed the grass-legume diet than for those fed the corn silage diets. The molar percentage of propionate for cows fed the corn silage diets was not different; however cows fed the Pro-Sil treated corn silage-medium grain diet had higher ($P < .01$) molar percentage of propionate than those fed the grass-legume silage diet. The molar percentage of butyrate was similar among treatments except for cows fed the low grain level which was higher ($P < .01$) than that for cows fed the grass-legume silage diet. The molar percentage of valerate was significantly ($P < .05$) higher for cows fed the urea-treated corn silage diet than for those fed the other diets. Cows fed the Pro-Sil-treated corn silage-medium grain diet had lower ($P < .01$) molar percentage of isovalerate than those

fed the urea-treated corn silage diet. The acetate to propionate ratio was not different ($P > .01$) among cows fed the corn silage diets, but was significantly ($P < .01$) lower for cows fed the Pro-Sil-treated corn silage-medium grain diet than for those fed the grass-legume silage diet. The non-glucogenic ratio followed the same trend as the acetate to propionate ratio.

Table 12. Rumen Volatile Fatty Acid Concentration and Molar Ratios, Ammonia-N and Blood Plasma Urea-N Levels of Cows Fed Experimental Diets (Expt. I)

	Diets				
Silage Grain Level Items	Grass-legume Medium	Urea-corn Medium	Pro-Sil-corn Medium	Pro-Sil-corn Low	SE±
Rumen ammonia-N (mg/100ml)	8.7	6.3	8.0	8.6	0.8
Plasma urea-N (mg/100ml)	14.4^{a}	13.2^{ab}	11.0^{c}	11.5^{bc}	0.6
Total VFA, mM/l	84.2	77.8	74.8	79.3	2.5
Individual VFA (molar %)					
Acetic	69.95^{A}	66.24^{B}	64.12^{B}	66.03^{B}	0.65
Propionic	18.05^{A}	19.18^{AB}	22.31^{B}	19.14^{AB}	0.78
Butyric	9.68^{A}	11.52^{AB}	11.52^{AB}	12.46^{B}	0.50
Isobutyric	0.65	0.62	0.47	0.62	0.07
Valeric	1.02^{a}	1.19^{b}	0.95^{a}	1.00^{a}	0.05
Isovaleric	0.93^{AB}	1.09^{A}	0.67^{B}	0.78^{AB}	0.07
Acetate:Propionate	3.9^{A}	3.5^{AB}	3.0^{B}	3.5^{AB}	0.13
Non-glucogenic ratio[1]	4.8^{a}	4.5^{ab}	4.0^{b}	4.6^{ab}	0.18

[1] Non-glucogenic ratio = (Acetate + 2 Butyrate + Valerate)/(Propionate + Valerate); (Ørskov, 1977).

^{AB}Means with different superscripts are significantly different ($P < .01$).

^{abc}Means with different superscripts are significantly different ($P < .05$).

EXPERIMENT II

Silage Chemical Composition: Treatment of the corn plant material with NPN before ensiling increased the nitrogen content of the silage by approximately 60% (Table 13). Non-protein nitrogen addition was again evident by the substantial increase in the proportion of total N that was soluble in both water and 0.15M NaCl solution. Lactic acid content was increased with NPN additions, especially in the Pro-Sil treated (1.7%) corn silage. The NPN-treated silages appeared to have higher total organic acid content and lower pH values than the untreated silage.

Digestibility and N Balance with Sheep: Silage DM intake and DM consumption per unit matabolic body weight by sheep was lower ($P < .05$) for the urea-treated corn (UC) silage than for the rest of the silages (Table 14); although the trend was similar, silage DM consumption per unit body weight was not different ($P > .05$) among treatments. Sheep fed the UC silage also gained less weight ($P > .05$) than those fed either of the other silages.

Silage DM digestibility was not different ($P > .05$) among treatments. Apparent CP digestibility was similar among the NPN-treated silages but was lower ($P < .05$) for the untreated corn (C) silage as expected because of its lower CP content. Apparent digestibility of ADF was higher ($P < .05$) for the Pro-Sil-treated (1.7%) corn silage than for the other silages; although the trend was similar, energy

Table 13. Chemical Composition of Experimental Silages (Expt. II)

Items	Corn Silages			
	Untreated	Urea-treated[1]	Pro-Sil-treated (1.3%)	Pro-Sil-treated[1] (1.7%)
Dry matter content, %	38.0	32.0	42.0	32.0
	% DM			
Crude protein	7.19	12.00	10.81	11.06
Acid-detergent fibre	23.78	25.24	22.59	23.99
Gross energy (Kcal/g)	4.40	4.39	4.35	4.39
Calcium	0.23	-	0.53	-
Phosphorus	0.22	-	0.31	-
Total organic acids	5.20	8.18	7.26	9.72
Lactic acid	3.85	4.72	4.70	7.73
Acetic acid	1.14	3.29	2.16	1.68
Butyric acid	0.21	0.17	0.40	0.31
pH	4.05	3.80	3.85	3.75
% total N				
Water-soluble N	55.1	64.2	63.7	62.5
Water-soluble NPN	35.0	40.9	45.1	44.7
0.15 NaCl-soluble N	42.3	58.4	56.0	55.7
Ammonia-N	3.5	4.0	4.5	4.6
Recovery of added N[2] (%)	-	100	100	96

[1]Stored in bunker silos.

[2]Based on the N content ensiled and the N content of the material removed from the silo.

Table 14. Average Daily Dry Matter Consumption and Apparent Digestibility Coefficients by Sheep Fed Experimental Silages (Expt. II)

Items	Corn Silages				
	Untreated	Urea-treated	Pro-Sil treated (1.3%)	Pro-Sil treated (1.7%)	SE±
Dry matter intake					
g/day	1210[a]	1082[b]	1343[a]	1263[a]	35
% body weight	2.17	2.08	2.18	2.00	0.14
$g/KgW^{3/4}$	59.6[a]	51.7[b]	62.9[a]	58.8[a]	1.8
Body weight gain (g/day)	230	150	260	210	38
Apparent digestibility coefficients %					
Dry matter	71.9	70.9	72.2	75.9	1.5
Crude protein	56.0[a]	72.1[b]	65.8[b]	71.8[b]	2.4
Acid-detergent fibre	52.5[a]	53.7[a]	56.1[a]	61.2[b]	1.4
Gross energy	72.8	71.9	73.7	76.8	1.4

[a,b]Means with different superscripts are significantly different ($P < .05$).

digestibility was not different ($P > .05$) among treatments.

Average daily N intake, N excreted and N absorbed were significantly ($P < .05$) increased with the NPN treatments (Table 15). The proportion of N consumed that was excreted in urine was lower ($P < .05$) for sheep fed the C silage compared to those fed the other silages. Fecal N losses as percent of N intake were significantly ($P < .05$) higher for sheep fed the C silage than those fed the other silages. The daily quantities of N retained and the proportion of N consumed that was retained were not different ($P > .05$) among treatments. Sheep fed the C silage retained more ($P < .05$) N absorbed than those fed the NPN-treated silages. Nitrogen utilization was not different ($P > .05$) among the NPN treatments.

Rumen fluid ammonia-N and blood plasma urea-N concentrations were not different ($P > .05$) among sheep fed the NPN-treated silages, but were lower ($P < .01$ and $P. < .05$ respectively) for sheep fed the C silage (Table 16). Total rumen VFA concentration was not different ($P > .05$) among treatments nor were the molar percentages of individual VFA. The acetate to propionate ratio was lower ($P < .05$) for sheep fed Pro-Sil-treated (1.7%) corn silage than those fed the C silage.

Feed Consumption by Dairy Cows: Cows fed the Pro-Sil diet consumed less ($P < .05$) silage DM than those fed either the Fababean or Fababean plus methionine diets (Table 17). Concentrate DM consumption was lower ($P < .05$) for cows fed

Table 15. Nitrogen Utilization by Sheep Fed Experimental Silages (Expt. II)

Items	Corn Silages				
	Untreated	Urea-treated	Pro-Sil treated (1.3%)	Pro-Sil treated (1.7%)	SE±
Nitrogen balance, g/day					
Ingested	14.3[a]	21.8[b]	22.2[b]	22.8[b]	1.2
Fecal	6.3	6.1	7.6	6.4	0.8
Urine	3.1[a]	8.9[b]	8.9[b]	8.7[b]	1.0
Total excreted	9.4[a]	15.1[b]	16.5[b]	15.1[b]	1.3
Absorbed (apparent)	8.0[a]	15.7[b]	14.6[b]	16.4[b]	1.5
Retained	4.9	6.7	5.7	7.7	1.2
Percent of intake					
Fecal	44.0[a]	27.9[b]	34.2[b]	28.2[b]	2.4
Urine	21.8[a]	40.8[b]	40.2[b]	38.2[b]	3.7
Absorbed (apparent)	56.0[a]	72.1[b]	65.8[b]	71.8[b]	2.4
Retained	34.2	31.3	25.7	33.8	4.1
Percent of apparently absorbed N retained.	61.2[a]	42.7[b]	39.0[b]	46.9[b]	4.3

[a,b]Means with different superscripts are significantly different ($P < .05$).

Table 16. Rumen Volatile Fatty Acid Concentration and Molar Ratios, Ammonia-N and Blood Plasma Urea-N Levels of Sheep Fed Experimental Silages (Expt. II)

Items	Corn Silages				
	Untreated	Urea-treated	Pro-Sil treated (1.3%)	Pro-Sil treated (1.7%)	SE±
Rumen ammonia-N (mg/100ml)	2.7^{A}	33.2^{B}	25.2^{B}	31.1^{B}	3.3
Plasma urea-N (mg/100ml)	1.6^{a}	13.0^{b}	11.3^{b}	9.6^{b}	2.1
Total VFA, mM/l	83.4	105.1	103.6	97.8	1.1
Individual VFA (molar %)					
Acetic	58.44	53.76	56.38	48.97	3.01
Propionic	18.99	24.86	23.30	29.01	3.57
Butyric	18.25	18.06	19.80	19.42	2.83
Isobutyric	0.50	0.72	1.05	0.80	0.22
Valeric	1.54	1.36	1.02	1.02	0.25
Isovaleric	0.68	1.24	0.55	0.48	0.18
Acetate:propionate	3.08^{a}	2.16^{ab}	2.42^{ab}	1.69^{b}	0.26

^{A,B}Means with different superscripts are significantly different ($P < .01$).

^{a,b}Means with different superscripts are significantly different ($P < .05$).

concentrate mixture P compared to those fed concentrate mixture M. Total DM consumption was not different ($P > .05$) among treatments but was slightly lower for cows fed the Pro-Sil diet. Methionine supplementation had little effect on either silage or concentrate DM consumption. Total DM consumption per unit body weight was lower ($P < .01$) for cows fed the Pro-Sil diet compared to those fed the Fababean plus methionine diet. The forage to concentrate ratio for all treatments was 48:52.

Total N consumption was higher ($P < .01$) for cows fed the Urea diet compared to those fed the other diets (Table 17). Cows fed the Pro-Sil diet consumed less ($P < .01$) N than those fed either the Fababean or Fababean plus methionine diet. Soluble N consumption was higher ($P < .01$) from the Urea diet than from either of the other diets. Cows fed the Pro-Sil diet consumed more ($P < .01$) soluble N than those fed either the Fababean or Fababean plus methionine diet. Insoluble N intake was less ($P < .01$) for cows receiving the Pro-Sil diet compared to cows receiving the other diets. Soluble N intake as a proportion of total N intake was lower ($P < .01$) for cows fed either the Fababean or Fababean plus methionine diet compared to that for cows fed the NPN-containing diets (Pro-Sil or Urea).

The daily intake of sulfur-containing amino acids was somewhat similar for cows fed the Pro-Sil and Fababean diets but was higher for cows fed either the Urea or Fababean plus methionine diets (Table 18). Supplementation with encapsulated

Table 17. Average Daily Dry Matter Consumption By Dairy Cows Fed Experimental Diets (Expt. II)

Items	Diets: Silage Concentrate mix	Pro-Sil corn P	untreated corn U	untreated corn M	untreated corn F	SE±
Dry matter intake (Kg/day)						
Silage		7.1^{a}	7.7^{ab}	8.1^{b}	8.0^{b}	0.2
Concentrate		9.7^{a}	10.6^{ab}	11.0^{b}	10.5^{ab}	0.2
Hay		1.9	1.9	1.9	1.9	-
Total		18.7	20.2	21.0	20.4	3.2
Total feed (% body weight)		3.14^{A}	3.38^{AB}	3.55^{B}	3.45^{AB}	0.07
Silage (% total feed)		38.0	38.1	38.6	39.2	-
Forage:concentrate		48:52	48:52	48:52	48:52	-
Composition of total ration						
Acid-detergent fibre, %		16.5	15.6	16.0	17.4	-
Gross energy (Mcal/Kg DMI)		4.39	4.01	4.01	3.98	-
Crude protein, %		12.8	15.3	13.3	13.3	-
Total N intake (g/day)		382^{A}	493^{B}	448^{C}	433^{C}	10
Soluble N		112^{A}	145^{B}	99^{C}	93^{C}	3
Insoluble N		270^{A}	348^{B}	349^{B}	340^{B}	8
Soluble N (% of intake)		29.4^{A}	29.5^{A}	22.0^{B}	21.6^{B}	0.3

^{A,B,C} Means with different superscripts are significantly different ($P < .01$).

^{a,b} Means with different superscripts are significantly different ($P < .05$).

Table 18. Average Daily Intake of Sulfur-Containing Amino Acids and Other Essential Amino Acids by Dairy Cows Fed Experimental Diets (Expt. II)*

Amino Acid	Diets				
	Silage Concentrate mix	Pro-Sil corn P	untreated corn U	M	F
	g/day				
Cystine		30.5	46.9	38.8	36.3
Methionine		34.9	41.5	44.2	29.3
Total S-aa[1]		65.4	88.4	83.0	65.6
Arginine		90.4	147.2	181.9	169.7
Histidine		40.9	55.1	66.6	62.7
Threonine		84.2	126.2	127.6	123.0
Isoleucine		83.0	129.9	130.4	128.9
Leucine		161.7	258.2	269.2	260.9
Lysine		84.2	125.3	148.9	140.6
Phenylalanine		109.2	166.3	166.4	158.7
Valine		110.4	169.8	179.1	169.3

*Values are based on single analysis of composite feeds and average feed consumption (Tables 3 and 17) and are not statistically analyzed.

[1]Sulfur-containing amino acids.

methionine in concentrate mixture M supplied approximately 15g methionine/day over the control (concentrate mixture F). The daily consumption of the other essential amino acids tended to be lower for cows receiving the Pro-Sil diet compared to those receiving either of the other diets.

Milk Yields and Composition: Average daily milk yield was not different ($P > .05$) among treatments (Table 19). Butterfat content was lower ($P < .05$) for cows fed the Pro-Sil diet compared to those fed the Fababean diet. Cows fed the Urea and Fababean plus methionine diets, though not different ($P > .05$) had butterfat levels intermediate to those fed either the Pro-Sil or Fababean diet. Milk crude protein content was not different ($P > .05$) among treatments. Supplementing the Fababean grain with methionine had no significant effect on milk fat or protein content. Average 4% FCM yield was not different ($P > .05$) among treatments, but tended to be higher for cows fed the Fababean and Fababean plus methionine diets. Milk fat and protein yield, SNF and total solids content were not different ($P > .05$) among treatments. Average milk yield as a percentage of pre-experimental milk yield (persistency) was not different ($P > .05$) among treatments.

Digestibility and N Balance Trial: Apparent digestibility of DM was higher ($P < .05$) for the Pro-Sil and Urea diets compared to the Fababean and Fababean plus methionine diets (Table 20). Apparent digestibilities of CP and ADF were not

Table 19. Milk Yields and Milk Composition of Cows Fed Experimental Diets (Expt. II)

Items	Diets				
	Silage Concentrate mix	Pro-Sil corn	untreated corn		
		P	U	M	F
					SE±
Milk yield (Kg/day)	28.8	27.6	28.6	27.9	2.4
Butterfat, %	3.15[a]	3.53[ab]	3.57[ab]	3.74[b]	0.12
4% Fat-corrected milk (Kg/day)	25.1	25.7	26.8	26.8	0.7
Protein, %	3.64	3.68	3.68	3.68	0.95
Solids-not-fat, %	8.70	8.63	8.67	8.68	0.06
Total Solids, %	11.85	12.16	12.24	12.42	0.13
Butterfat yield (Kg/day)	0.91	0.97	1.02	1.04	0.04
Protein yield (Kg/day)	1.05	1.02	1.05	1.03	0.04
Milk persistency[1], %	93.8	89.9	98.2	89.2	3.0

[a,b]Means with different superscripts are significantly different ($P < .05$).

[1]Milk persistency = 100 x (Treatment milk yield/Pre-experimental milk yield).

Table 20. Apparent Digestibility Coefficients and Digestible Nutrient Intake of Cows Fed Experimental Diets (Expt. II)

Items	Diets				
	Silage	Pro-Sil corn	untreated corn		
	Concentrate mix	P	U	M	F	SE±
Apparent digestibility coefficients, %					
Dry matter	73.5[a]	74.2[a]	68.3[b]	69.3[b]	1.1
Crude protein	74.4	73.9	66.0	66.8	2.2
Acid-detergent fibre	47.1	49.8	36.7	38.6	3.8
Gross energy	69.7[ab]	74.8[a]	68.4[b]	73.9[a]	1.2
Digestible nutrient intake					
Crude protein (Kg/day)	1.68	2.01	1.65	1.70	0.09
Acid-detergent fibre (Kg/day)	1.45	1.57	1.23	1.37	0.15
Energy (Mcal/Kg DMI/day)	3.06	3.00	2.74	2.94	2.30
CP intake/NRC requirements[1] (%)	86.5	136.2	97.7	95.5	-
DE intake/NRC requirements (%)	102.7	108.6	102.1	104.7	-

[a,b]Means with different superscripts are significantly different ($P < .05$).

[1]Protein and DE requirements for maintenance plus milk production estimated from NRC (1978) Tables.

different ($P > .05$) among treatments, but tended to be slightly lower for the Fababean and Fababean plus methionine diets. Apparent digestibility of energy was lower ($P < .05$) for cows receiving the Fababean plus methionine diet than for cows fed either the Urea or Fababean diet. The daily consumption of digestible CP, ADF and energy was not different ($P > .05$) among treatments.

Cows fed the Pro-Sil diet consumed less ($P < .01$) N than those fed the Urea diet (Table 21). Cows receiving either the Fababean or Fababean plus methionine diet consumed more ($P < .05$) N than those fed the Pro-Sil diet. The Pro-Sil diet resulted in less ($P < .01$) insoluble N consumption than either of the other diets.

The mean quantity of nitrogen excreted in feces was lower ($P < .05$) for cows fed the Pro-Sil diet than for those fed the Fababean diets. Cows fed the Urea diet absorbed and excreted more N in urine than the rest of the cows but the difference was not significant ($P > .05$). Average daily N excreted in milk, productive N and retained N were not different ($P > .05$) among treatments. The proportion of N consumed that was excreted in milk was lower ($P < .05$) for cows fed the Urea diet than those fed either of the other diets. Fecal N and urinal N losses as a percentage of intake were not different ($P > .05$) among treatments nor were productive N and retained N. The efficiency of conversion of absorbed N into milk was not different ($P > .05$) among treatments. Methionine supplementation had little effect on

Table 21. Nitrogen Intake and Utilization and Body Weight Change of Cows Fed Experimental Diets (Expt. II)

Items	Diets: Silage Concentrate mix	Pro-Sil corn P	untreated corn U	untreated corn M	untreated corn F	SE±
Nitrogen balance, g/day						
Ingested		361^{Aa}	433^{Bb}	400^{ABb}	406^{ABb}	12
Soluble N		108^{ABa}	128^{Bb}	88^{Ac}	87^{Ac}	4
Insoluble N		253^{A}	305^{B}	312^{B}	319^{B}	8
Fecal		92^{a}	112^{ab}	136^{b}	134^{b}	9
Urine		88	102	81	84	7
Milk		137	135	144	142	3
Absorbed (apparent)		269	321	264	272	15
Retained[1]		44	84	39	46	20
Productive[2]		181	219	183	188	20
Percent of N Intake						
Soluble N		29.8^{A}	29.6^{A}	22.0^{B}	21.4^{B}	0.3
Fecal		25.6	26.1	33.9	33.2	2.2
Urine		26.5	23.5	20.3	20.8	2.0
Milk		38.2^{b}	31.2^{a}	36.0^{b}	34.9^{b}	0.8
Absorbed (apparent)		74.4	73.9	66.0	66.8	2.2
Retained		11.8	19.2	9.7	11.1	4.5
Productive		50.0	50.4	45.7	46.0	4.7

CONTINUED .....

Table 21. CONTINUED

Items	Diets: Silage Concentrate mix	Pro-Sil corn: P	untreated corn: U	untreated corn: M	untreated corn: F	SE±
Percent of apparently absorbed N						
Urine		33.1	32.2	30.8	31.5	3.8
Milk		51.3	42.6	54.5	52.2	3.4
Retained		15.6	25.2	14.7	16.3	6.0
Productive		66.9	67.7	69.2	68.5	3.8
Weight gain, Kg/day		0.27	0.22	0.43	0.30	0.14

[1]Retained N = N intake - (Fecal N + Urinal N + Milk N).

[2]Productive N = Milk N + Retained N.

[a,b,c]Means with different superscripts are significantly different ($P < .05$).

[A,B]Means with different superscripts are significantly different ($P < .01$).

nitrogen utilization. Average daily weight gain was not different ($P > .05$) among treatments.

Rumen fluid VFA, ammonia-N and plasma Urea-N levels: Rumen fluid NH_3-N and blood plasma Urea-N concentrations were not different ($P > .05$) among treatments (Table 22). Total rumen VFA concentrations were not significantly ($P > .05$) affected by treatment. The molar concentrations of individual VFA were similar among treatments except for isobutyrate which was higher ($P < .05$) for cows fed the Urea diet compared to those fed either the Pro-Sil or Fababean plus methionine diet. The acetate to propionate ratio and NGR were not different ($P > .05$) among treatments.

Plasma Amino Acids: Feeding encapsulated methionine did not significantly ($P > .05$) affect either the plasma methionine levels or Met/Val ratio (Table 23). Plasma leucine concentration was higher ($P < .05$) for cows fed the Pro-Sil diet than for those fed either of the other diets. Cows fed the Fababean diet had higher ($P < .05$) plasma tyrosine concentration than cows fed either of the other diets. There were no significant ($P > .05$) differences among treatments for the rest of the analyzed essential and non-essential amino acids.

Table 22. Rumen Volatile Fatty Acids Concentration and Molar Ratios, Ammonia-N and Blood Plasma Urea-N Levels of Cows Fed Experimental Diets (Expt. II)

	Diets				
	Silage	Pro-Sil corn	untreated corn		
Items	Concentrate mix	P	U	M	F	SE±
Rumen ammonia-N (mg/100ml)		8.50	5.94	5.58	6.47	0.99
Plasma urea-N (mg/100ml)		11.78	14.88	11.56	12.01	0.76
Total VFA, mM/l		96.0	96.0	95.1	82.2	10.8
Individual VFA (molar %)						
Acetic		59.46	60.07	57.90	60.76	2.36
Propionic		25.52	23.72	27.08	21.79	2.68
Butyric		11.28	12.63	11.05	13.91	0.77
Isobutyric		0.58[a]	0.82[b]	0.52[a]	0.67[ab]	0.06
Valeric		2.06	1.65	2.41	1.54	0.36
Isovaleric		1.10	1.11	1.04	1.33	0.14
Acetate:propionate		2.3	2.5	2.1	2.8	0.3
Non-glucogenic ratio[1]		3.0	3.4	2.8	3.9	0.5

[1]Non-glucogenic ratio = (Acetate + 2 Butyrate + Valerate)/(Propionate + Valerate); (Ørskov, 1977).

[a,b]Means with different superscripts are significantly different ($P < .05$).

Table 23. Plasma Free Amino Acid Concentrations of Cows Fed Experimental Diets (Expt. II)

Amino Acid	Diets: Silage Concentrate mix	Pro-Sil corn P	untreated corn U	untreated corn M	untreated corn F	SE±
			µmole/100ml			
Serine	16.38	19.70	24.43	14.72	2.92
Glutamic acid	20.67	18.72	19.35	18.14	0.24
Proline	7.46	7.28	8.20	7.31	0.23
Glycine	26.24	25.24	33.93	27.58	3.00
Alanine	26.77	24.84	26.88	21.14	2.71
Citrulline	9.44	7.82	9.73	8.53	1.25
Tyrosine	2.94[a]	3.04[a]	3.08[a]	3.78[b]	0.19
Threonine	10.72	9.28	10.80	9.49	0.54
Isoleucine	10.53	11.44	11.18	12.94	0.64
Leucine	13.92[a]	11.66[b]	11.91[b]	11.04[b]	0.59
Methionine	1.78	1.50	1.71	1.88	0.19
Valine	13.30	14.58	14.58	17.20	0.96
Met/Val	0.144	0.119	0.139	0.123	0.069

[a,b]Means with different superscripts are significantly different ($P < .05$).

DISCUSSION

Silage Chemical Composition: The addition of either urea (0.5% wet basis) or Pro-Sil (2.2% wet basis) raised the N content of the corn plant material from approximately 1.1% to 2% in experiment I (Table 4). This represents an increase in N content of about 80%. However, in experiment II, the increase in N content as a result of NPN treatment, was lower compared to that obtained in experiment I (Table 13). This was because lower rates of Pro-Sil (1.3% and 1.7% wet basis) were used.

These results are in general agreement with many experiments as reviewed by Ely (1978) in which urea has been added to low protein silages at a level of 0.5% to improve the protein content of the feed. Ammonia, applied in various forms (Ely, 1978) has also improved the N content of silage. But additions of ammonia are hard to calculate as some ammonia is lost to the atmosphere during addition. Much work has been done with Pro-Sil as an addition to corn silage (Ely, 1978). Pro-Sil is normally applied at 2.25% (wet basis). Rates of recovery of applied N ranged from 100% (experiment II) to 95% (experiment I). These rates compare very well with the range of 100% (Cash et al., 1971; Henderson et al., 1972a,b) to 80% (Huber and Thomas, 1971; Phipps and Fulford, 1977). Approximately 50% of the urea added to corn silage is hydrolyzed to ammonia and negligible losses of N occur providing the silage contains a minimum of

30% DM at ensiling (Cash, 1975). In both experiments I and II, all the applied N in urea was accounted for by the increase in N content of the silage.

In both experiments I and II, NPN addition increased N solubility in either water or 0.15M NaCl solution compared to no additions (grass-legume and untreated corn silages) (Tables 4 and 13). Ammonia-N content was also increased with NPN treatments. However, the levels of ammonia-N obtained in both experiments I and II (3.0 - 5.6%) were much lower than the levels of 9 to 11% of total N observed in high lactate silages(McDonald and Edwards, 1976; McDonald and Whittenbury, 1973). Compared to control, NPN treatment increased the water insoluble N content of the silages (Table 13). This is in agreement with previous work (Phipps and Fulford, 1977; Huber et al., 1973; Huber and Santana, 1972; Huber and Thomas, 1971). The increase in water insoluble N content appears related to decreased proteolysis of the original plant material (Bergen et al., 1974) or increased production of microbial protein as suggested by several workers (Coppock and Stone, 1968; Owens et al., 1970; Cash et al., 1971; Huber et al., 1979). In both experiments I and II, the urea-treated corn silages contained more water-insoluble N content than the Pro-Sil-treated corn silages (Tables 4 and 13). This is in contrast to results reported by Huber (1975) in which ammonia-treated corn silages had higher water-insoluble N contents than either urea-treated or untreated corn silages.

The high protein solubility in silages is attributed to proteolysis by plant enzymes and protein solubilization by acids during storage (Bergen et al., 1974; Pitchard and Van Soest, 1977). Fermentation of forages tends to increase the NPN (water-soluble) and the unavailable N fractions at the expense of the insoluble N fraction (Pitchard and Van Soest, 1978). Degradation of plant protein to water soluble, non-protein nitrogenous compounds may be quite extensive in whole corn plant material. Bergen et al., (1974) and Demarquilly and Andrieu (1973) found that 35% DM corn silage contained over 40% of total N in water soluble form. The extent of degradation may be influenced by stage of maturity of the crop at harvest, since Geasler (1970) found that mature corn plant material ensiled at 48% and 59.6% contained only 30.3% and 26.1% of total N in the water-soluble form respectively. The higher water-soluble N contents obtained in the present studies would be expected since the corn plant material contained less DM% (31 to 42) than that used by Geasler (1970).

The NPN-treated corn silages had higher lactic acid content compared to untreated corn or grass-legume silages (Tables 4 and 13). Increased levels of lactic acid have been recorded in NPN-treated corn silages (Henderson et al., 1971; Henderson et al., 1972b; Honig and Zimmer, 1975; Soper and Owen, 1977). The increased lactic acid content is due to a buffering effect of ammonia or ammonium salts formed during fermentation (Huber, 1975). Non-protein nitrogen

treatment also increased total organic acid content of the silages compared to untreated or grass-legume silage. The acetic acid content of the silages was also increased with NPN-treatment, in agreement with results of Phipps and Fulford, (1977) and Huber and Thomas, (1971). However other workers have not reported increased levels of acetate in NPN-treated corn silages (Henderson and Geasler, 1970; Henderson et al., 1972b; Soper and Owen, 1977).

Digestibility and Nitrogen balance with sheep: In experiment I, no significant ($P > .05$) differences were noted among treatments for silage DM consumption (Table 5). Silage DM consumption was significantly ($P < .05$) less for the urea-treated corn silage compared to the rest of the silages in experiment II (Table 14). Silage DM intake is positively associated with silage DM% over a wide range of DM contents (Gordon et al., 1965; Ward et al., 1966; Thomas et al., 1961; Hawkins et al., 1970; Wilkins et al., 1971). This could partly explain the low intake of urea-treated corn silage (32% DM) observed in experiment II compared to either untreated corn silage (38% DM) or Pro-Sil-treated (1.3%) corn silage (42% DM). Moisture content per se does not relate to depression in silage intake (Baile and Forbes, 1974; Harris et al., 1966; Thomas et al., 1961). It is more probable that metabolic constituents produced or altered during fermentation of wet material may reduce silage intake (Harris et al., 1966).

Research with both grass silage (McLeod et al., 1970) and corn silage (Thomas and Wilkinson, 1975) demonstrated that the free acid content of the ensiled product was associated with reduced voluntary consumption compared to material which had been partially neutralized with sodium bicarbonate. No such relationship was noted in experiment I. The higher acetate content in urea-treated corn silage compared to the other silages (Table 13) could have caused the significant ($P < .05$) depression in silage DM intake observed in experiment II. Allen and Henderson (1972) depressed silage DM intake by adding acetate to corn silage. Infusions of acetate into the rumen have resulted in reduced voluntary feed consumption (Rook et al., 1963; Ullyatt, 1965). But Hutchinson and Wilkins (1971) concluded that high acetate level per se was unlikely to result in a low intake of silage.

In both experiment I and II, silage DM consumption from NPN-treated silages was not affected by the higher levels of ammonia-N and water-soluble-N contents compared to untreated corn or grass-legume silage. The products of protein breakdown during silage fermentation may influence the taste and physiological effect of silage (Voss, 1966). Wilkins et al., (1971) reported that ammonia-N content accounted for 77% of the depression in grass silage DM intake by sheep. Griffiths and Wilson (1976) observed rapid metabolism of amino acids in the rumen of heifers given grass silage suggesting that free amino acids in silage were not likely

to affect voluntary intake.

Despite its low N content (1.15%N) untreated corn silage was consumed as well as the NPN-treated corn silages which had higher N contents in experiment II (Table 14). Wilkins et al., (1971) demonstrated a positive relationship between silage DM intake and silage N content. The low protein status of the animal has been implicated with decreased silage DM consumption (Thomson, 1968). Abomasal infusions of casein increased the voluntary intake of sheep fed dried forages containing 0.7% or less N (Egan, 1965; Weston, 1967) but failed to increase grass silage DM consumption (Hutchinson et al., 1971). Hutchinson et al., (1971) concluded that the positive relationship found between silage intake and silage nitrogen content (Wilkins et al., 1971) is unlikely to be due to a low nitrogen status in sheep fed all silage rations.

Apparent digestibilities of DM, crude protein and gross energy (Table 5) were lower ($P < .05$) for sheep fed grass-legume silage than those fed either Pro-Sil-treated or urea-treated corn silage, probably due to the higher ADF level in the grass-legume silage (Table 4). Grass and hay crop silages have N contents in excess of available digestible energy (Bergen, 1975; McDonald and Edwards, 1976), which would also explain the results in the present experiment.

In experiment II, apparent digestibility of crude protein was lower ($P < .05$) for sheep fed the untreated corn silage than for those fed the NPN-treated corn silages even

though apparent digestibility of DM was not different ($P > .05$) (Table 14). This is due to differences in N intake (Table 15) and similar observations were reported by Bergen (1975) and Buchanan-Smith and Yao (1978). The low nitrogen intake by sheep fed the untreated corn silage resulted in a rumen ammonia-N level (Table 16) which was less than the minimum level suggested to support maximal growth rates of rumen microorganisms (Satter and Slyter, 1974; Roffler et al., 1976). This low rumen activity may have resulted in the significantly ($P < .05$) lower ADF digestibility (Table 14).

No significant ($P > .05$) differences were noted among the treatments of experiment I for nitrogen intake, nitrogen excreted in feces and urine or nitrogen retained (Table 6). In experiment II, significant ($P < .05$) differences were found among treatments for N intake, N excreted in urine and N absorbed (Table 15). This would be expected since sheep fed the untreated corn silage consumed less N. Improved nitrogen utilization by sheep fed the untreated corn silage compared to those fed the NPN-treated corn silage could be due to lower N intake, since efficiency of utilization of N is known to be increased with lower N intake (Mitchell, 1964). Recycled urea could have met some of the need for N by rumen bacteria in sheep fed the untreated corn silage. A large amount of net influx of N into the rumen has been reported with low N diets, while N efflux due to absorption is common with high N diets (Hogan

and Weston, 1970; Hume et al., 1970; Allen and Miller, 1972; Leibholz and Hartman, 1972).

Both in vitro studies (Bergen et al., 1974) and in vivo studies (Buchanan-Smith and Yao, 1978) suggested that the NPN in corn silage is of a slow release form and may in fact limit microbial fermentation in animals fed solely corn silage. In the present studies, this fact would have been demonstrated by low rumen ammonia levels and low DM and (or) fiber digestibility. Except for sheep fed untreated corn silage, these responses were not obtained.

In experiment II, plasma urea-N levels were higher ($P < .05$) for sheep fed the NPN-treated corn silage than those fed the untreated corn silage (Table 16). Plasma urea-N is a major determinant of the N excreted in urine (Thornton and Wilson, 1972) which partly explains the significantly ($P < .05$) higher urinal N losses in sheep fed the NPN-treated corn silages. Increased lactic acid intake from the NPN-treated silages could also have contributed to the increased urinary N excretion. Wilkinson et al., (1976a) reported increased urinary N excretion in young calves fed frozen or ensiled corn plant material supplemented with a mixture of lactic and acetic acids and suggested a detrimental effect of acids on N balance via increased urinary ammonia excretion and acidification of the urine in response to the acid load. However, this was not true in Experiment I (Table 6).

In experiment I, the molar proportion of acetate decreased ($P < .05$) and that of butyrate increased ($P < .10$) in sheep

fed the Pro-Sil-treated corn silage compared to those fed the grass-legume silage (Table 7). Differences in ADF contents of the silage (Table 4) could partly explain this since rumen acetate is linearly related to crude fibre content of the diet (Anderson and Jackson, 1971). No significant ($P > .05$) differences were observed among the treatments of experiment II for total ruminal VFA concentration and molar proportions of individual VFA (Table 16). Honig and Rohr (1973) showed a negative correlation between lactic acid intake and acetate to propionate ratio in the rumen contents of dairy cows. Donaldson and Edwards, (1977) obtained similar results in sheep fed grass silage. These results are evidence for conversion of silage lactic acid to propionate in the rumen. This might have been the case in the present studies in sheep fed the NPN-treated corn silages with comparatively higher lactic acid contents than either grass-legume or untreated corn silages.

Feed consumption by dairy cows: No significant ($P > .05$) differences were noted among the treatments of experiment I for silage DM consumption (Table 8). Concentrate DM consumption was significantly ($P < .05$) reduced with the low level compared to the medium level of grain feeding by experimental design. However, this decrease in concentrate consumption was partly compensated with a slight increase in silage DM consumption. Total DM consumption was significantly ($P < .05$) decreased with the low level compared to the medium level of grain feeding. Bull et al., (1976)

reported increased DM consumption by dairy cows from diets of high caloric density compared to diets of low caloric density. This would be expected from the physical capacity of the digestive tract probably being limiting with dilute (low caloric density) diets (Baumgardt, 1970). As the proportion of roughage in the diet was reduced, there was a linear decrease in the importance of distension of the reticulo-rumen in the regulation of food intake (Bines and Davey, 1970). In experiment II, there were significant ($P < .05$) differences among treatments for silage DM consumption (Table 17). No significant ($P > .05$) differences among treatments were observed for total DM consumption.

Generally DM consumption by cows fed Pro-Sil or urea-treated corn silage has been higher than for those fed untreated corn silage (Huber, 1975; Ely, 1978). But Bull and Little (1975) reported reduced silage DM intake by cows in late lactation with Pro-Sil-treated compared to untreated corn silage. Owen (1975) reported decreased consumption of Pro-Sil-treated compared to untreated corn silage when included in high fiber diets.

The reduced intake of Pro-Sil-treated compared to untreated corn silage in experiment II may have been due to higher free-acid content in the Pro-Sil-treated corn silage (Table 13). Research with both grass silage (McLeod et al., 1970) and corn silage (Thomas and Wilkinson, 1975) has demonstrated an association of free-acid content of the

ensiled product with reduced voluntary consumption. Wilkinson et al., (1976a) reported reduced DM intake by young calves fed frozen or ensiled corn plant material with addition of a mixture of acetic and lactic acids. Extensive degradation of plant protein to non-protein nitrogenous compounds, especially water-soluble N (WSN) has been demonstrated in the ensiled corn plant material (Bergen et al., 1974; Demarquilly and Andrieu, 1973; Geasler, 1970). Geasler, (1970) reported a highly negative relationship between WSN content of corn silage and voluntary DM intake by steers but other workers (Wilkinson et al., 1976a; Bergen et al., 1974; Buchanan-Smith and Yao, 1978) failed to demonstrate the above relationship. Compared to untreated corn silage, Pro-Sil-treated corn silage had higher N in the water soluble form (Table 13) and this could have led to its reduced consumption in experiment II.

The low intake of crude protein in experiment II (Table 17) may have contributed to the lower intakes of silage DM and total DM for cows fed the Pro-Sil-treated corn silage diet. Feeding diets that contain less than 10%CP depressed feed intake of dairy cows (Elliot, 1967; Wohlt and Clark, 1978). If a dietary deficiency of N limits the rate of fermentation, voluntary feed intake and organic matter digestibility will both be depressed (Campling et al., 1961; Mehrez and Ørskov, 1978).

In experiment II, inclusion of fababean up to 42% in the concentrate mixture did not affect concentrate DM

consumption compared to concentrate mixtures containing SBM or Urea (P and U) (Table 17). Ingalls and McKirdy (1974) reported no effect on feed intake when 17 or 35% fababeans were added to a dairy concentrate. Hansen and Andersen (1972) obtained similar results with concentrates containing 60% fababeans.

Adding encapsulated methionine (0.6% of concentrate mixture) to supply approximately 15g methionine/day appeared to have little effect ($P > .05$) on feed consumption. These results are in general agreement with other results reported by Williams et al., (1970) and Broderick et al., (1970).

Ration Digestibility: No significant ($P > .05$) differences were observed among the treatments of experiment I for crude protein and ADF digestibility (Table 10). Apparent digestibilities of DM and gross energy were lower ($P < .05$) for the grass-legume silage diet compared to the corn silage diets in accordance with the results obtained with sheep (Table 5). In experiment II, apparent digestibility of DM was significantly ($P < .05$) decreased in fababean-containing diets compared to the other diets (Table 20). The decreasing effect of fababeans on ration DM digestibility could be due to the presence of condensed tannins in fababeans (Marquardt et al., 1977) which have been shown to reduce DM digestibility (McLeod, 1974; Buckley, 1978).

No significant ($P > .05$) differences were observed among the treatments of experiments I and II for digestible CP, ADF and energy intakes (Tables 10 and 20). Nitrogen and

digestible energy requirements for maintenance and production of the cows used in both experiments I and II were estimated using NRC (1978) Tables (Tables 10 and 20). In experiment I, N and digestible energy intake by cows were in excess of the estimated requirements (Table 10). In experiment II, N intake was below the estimated requirements for all cows except those fed the Urea diet (Table 20). Digestible energy intake was in excess of the estimated requirements.

Milk Yields and Composition: The results of experiment I showed no significant ($P > .05$) differences among treatments for milk and FCM yields and milk composition (Table 9). Except for fat content, no significant ($P > .05$) differences were noted among treatments of experiment II for milk yield and composition (Table 19). The amount of protein and energy consumed by lactating dairy cows affects milk yield and composition and change of body weight (Gordon and Forbes, 1970; Broster, 1972; Paquay et al., 1973). Recommendations for daily protein requirements (NRC, 1978) of lactating cows are based largely on milk production, milk composition and body weight but don't take into account the different portions (ascending or descending) of the lactation curve. Nitrogen and DE were not limiting production of milk and milk components by cows in experiment I since the cows were consuming N and DE in excess of their estimated requirements (Table 10). In experiment II, N intake was limiting in all but the Urea treatment (Table 20). Milk yields were however

not different (P > .05) among treatments. So the cows must have mobilized protein from body tissue to meet the requirements for lactation (Coppock et al., 1968; Botts et al., 1979).

A key objective in NPN utilization has been to increase total ration NPN without decreasing milk yields in high producing cows. The increase in water insoluble N resulting from ammonia treatment of corn silage (Huber, 1975) may permit the feeding of higher levels of NPN in concentrate without depressing milk yields than is possible with urea-treated corn silage. Milk yields of cows receiving ammoniated silage were maintained higher than those of cows on urea-treated silage when 1.4 to 1.5% urea was added to concentrates fed with both silages (Huber and Bucholtz, 1974). The results of experiment I showed no significant (P > .05) differences between milk yields of cows fed either Pro-Sil or urea-treated corn silage (Table 9). Other workers (Lichtenwalner et al., 1972; Huber et al., 1973; Huber and Bucholtz, 1974) reported slightly higher milk yields for cows fed ammonia-treated compared to urea-treated corn silage. Milk yield and milk composition were maintained when Pro-Sil-treated corn silage replaced some concentrate with the low level of grain feeding in experiment I (Table 9). Phipps and Cramp (1978) obtained similar results when Pro-Sil-treated corn silage replaced the groundnut component of a dairy concentrate.

Although there were significant (P < .05 and P < .01

respectively) differences in N solubility of the diets in both experiments I and II, milk yields and milk protein content were not different ($P > .05$) (Tables 9 and 19). Majdoub et al., (1978) reported a negative correlation of soluble N intake with milk yield and milk protein yields with diets of 22 and 42% soluble N. Aitchison et al., (1976) suggested that N solubility was important because the soluble N fraction contributed less to net available N for absorption. Dingley et al., (1975) reported that amino acid supply to the udder was influenced by solubility of dietary protein.

Milk fat content was particularly low in experiment II compared to the results of experiment I (Tables 9 and 19). This would be expected (Bauman et al., 1971) with the ADF levels of the rations (Table 17) which were below the NRC (1978) recommended levels. The milk fat content was higher ($P < .05$) for cows fed the Fababean diet compared to that for cows fed the Pro-Sil diet (Table 19). These results are partly consistent with the ADF levels and the acetate to propionate ratios (Tables 17 and 22). Ingalls and McKirdy (1974) reported higher ($P < .01$) butterfat test for cows fed a diet containing soybean meal or rapeseed meal under fat-depressed conditions somewhat similar to the present studies. Hansen and Andersen, (1972) reported increased milk fat and decreased milk protein when a concentrate containing 60% fababeans was fed to dairy cows. Feeding encapsulated methionine had no effect on milk fat content, in agreement with previous reports (Williams et al., 1970;

Broderick et al., 1970).

Nitrogen Balance: Nitrogen intake, N excreted or N retained were not different ($P > .05$) among the treatments of experiment I (Table 11). The percentage of dietary N recovered in milk was lower ($P < .05$) for cows fed the grass-legume diet compared to those fed the Pro-Sil-treated corn diets. This was probably due to the slightly higher ($P > .05$) N intake for cows fed the grass-legume diet. There were significant ($P < .05$) differences among the treatments of experiment II for the total quantities of dietary N and fecal N (Table 21). Cows fed the Pro-Sil diet consumed less ($P < .05$) N than those fed either of the other diets. Fecal N was less ($p < .05$) for cows fed the Pro-Sil diet than those fed the Fababean and Fababean plus methionine diets because of the lower ($P < .05$) N intake. The slightly lower ($P > .05$) DM intake by the same cows (Table 17) could also account for differences in fecal N since fecal N is highly correlated with DM intake (Stallcup et al., 1975).

No significant ($P > .05$) differences were noted among the treatments of both experiment I and II for the percentage of absorbed N recovered in milk and urine or retained despite significant ($P < .05$ and $P < .01$, respectively) differences in N solubilities of the diets (Tables 8 and 17). Other workers (Wohlt et al., 1976; Majdoub et al., 1978; Aitchison et al., 1976) reported improved N utilization with diets of low compared to high protein solubility. MacGregor

et al., (1978) using an in vitro system showed marked differences between amino acid profiles of the total protein and the amino acid profile of the insoluble protein in commonly fed livestock feeds. If these differences do exist in vivo, then N solubility may be a poor indicator of the quantities of amino acids reaching and being absorbed from the small intestines.

A mathematical model described by Mertens (1975) and as applied by Aitchison et al., (1976) was used to determine the utilization coefficients of soluble and insoluble N components of total N in experiment I. Insoluble N can be utilized either via rumen degradation and subsequently incorporated into microbial N or through direct postruminal utilization of the protein that escapes rumen degradation whereas soluble N can be utilized only via incorporation into microbial N (Aitchison et al., 1976). The model as outlined by Aitchison et al., (1976) is essentially a multiple regression model:

$$Y = a + b_1X_1 + b_2X_2 + e$$

where Y is the utilizable (productive) or net available N (g/day) (i.e. nitrogen which is ingested, but not excreted in feces or urine), X_1 is the insoluble N (INSOLN) consumed (g/day), X_2 is the soluble N (SOLN) consumed (g/day) and e is a random error. The nutritional interpretations of this model are: a is the total endogenous N loss of the animal (i.e. metabolic fecal N + endogenous urinary N), b_1 is the INSOLN utilization coefficient and b_2 is the SOLN utilization

coefficient. The regression equation obtained in the present experiment was:

$$Y = -98.8 + .69X_1 + .44X_2 \quad (r = .99, P < .05)$$

These results confirm the results of Aitchison et al., (1976) which showed large differences between soluble and insoluble N utilization and agree with the conclusions of Dingley et al., (1975) and Sniffen, (1974). Aitchison et al., (1976) also observed decreasing utilization of total N with increasing soluble N content of the diet. But relatively constant utilization coefficients of N were obtained in 3 trials in which diets of 12, 13 and 15% CP were fed to lactating dairy cows. According to the hypothesis of Satter and Roffler, (1975) greater utilization of N would be expected in the lower protein diets and poorer utilization in the higher protein diets. However recent results of Edwards and Bartley, (1979) refute the hypothesis of zero urea utilization by dairy cattle if the ration already contains 12 - 13% CP from natural sources. According to Aitchison et al., (1976), much of the soluble N pool, including urea-N is washed out of the rumen unused as suggested by earlier studies (Hecker, 1971; Nolan and Leng, 1972). The amount of soluble N that is used remains fairly constant in high producing dairy cattle (Aitchison et al., 1976). The utilization of soluble N may be more dependent upon DM and water intake and less dependent upon the CP content or production level of the cow as has been suggested.

In experiment I, no significant ($P > .05$) differences

were noted among treatments for body weight gain, but cows fed the low grain level lost weight (Table 11). This would be expected from the N intake and balance data (Table 11). Negative N balance in early lactation occurs as the demand for milk N exceeds the available amino acid supply and demonstrates the ability of the cow to mobilize protein from body reserves for milk secretion (Botts et al., 1979; Paquay et al., 1972). Botts et al., (1979) estimated that the lactating dairy cow has a valuable protein reserve ranging from 25 to 27% of body protein. In experiment II, no significant ($P > .05$) differences were observed among treatments for body weight gain (Table 21) despite N intake being below estimated NRC (1978) requirements (Table 20).

Ruminal ammonia-N, VFA and Plasma Urea-N: In experiment I, there were no significant ($P > .05$) differences among treatments for rumen ammonia (RAN) concentration (Table 12); however significant ($P < .05$) differences were noted for blood plasma urea (BUN) concentration (Table 12). No significant ($P < .05$) differences were observed among the treatments of experiment II for RAN and BUN (Table 22). The RAN concentration that results in maximal microbial growth is unresolved. Satter and Slyter (1974) recommended 5 mg/100 ml rumen fluid as the upper limit. Other workers (Helmer et al., 1970; Mehrez and Ørskov, 1976; Miller, 1973) indicated that more than 5 mg/100 ml rumen fluid may be required for maximum digestion of energy. Rumen ammonia concentration

can be affected by several factors including form of grain energy (Colenbrander, 1968), percent dietary protein (Roffler and Satter, 1975), system of feeding (Coppock et al., 1976) and solubility of dietary N (Wohlt et al., 1973). The data in the present studies would indicate that the RAN concentration was enough in experiment I but somewhat marginal for some treatments of experiment II for maximum microbial production.

Blood urea nitrogen concentration was shown (Lewis, 1957) to be a sensitive indicator of changes in RAN through conversion of the absorbed ammonia to urea by the liver. Preston et al., (1965) noted a correlation of BUN with dietary protein over a large range of diets fed to growing lambs. Dietary protein percentage in diets of lactating cows also has directly affected BUN (Treacher et al., 1976; Manston et al., 1975). The results of the present studies are in general agreement with the above observations.

Total ruminal VFA concentration was not different ($P > .05$) among the treatments of experiment I (Table 12). There were highly significant ($P < .01$) differences among the treatments for the molar percentages of acetate, propionate, butyrate, isovalerate and acetate:propionate ratio. Significant ($P < .05$) differences also existed among treatments for the molar percentage of valerate and the non-glucogenic ratio. However milk butterfat and solids-not-fat contents were not affected by these differences. In experiment II, no significant ($P > .05$) differences were noted for

either total ruminal VFA concentration or individual VFA molar concentrations except for isobutyrate among treatments (Table 22). However significant ($P < .05$) differences were observed among the treatments for butterfat test (Table 19). Van Soest (1963) reviewed the causes of low milk fat test. Fat-depressing diets have a low acetate to propionate ratio which was true for all treatments in experiment II (Table 22).

Investigations into the low milk fat problem have centred around three theories. Balch et al., (1955) suggested acetate deficiency, Van Soest and Allen (1959) proposed that an increase in production of propionate decreased production and availability of ketone bodies especially β-hydroxy butyric acid (BHBA) for milk fat synthesis. McClymount and Vallance (1962) postulated that the glucogenic response during high propionate production induces the release of insulin which supresses mobilization of fat from tissues thereby causing a decline in blood lipids required for milk fat synthesis. Reports by Bauman et al., (1971), Davis, (1967) and Palmquist et al., (1969) have refuted the acetate and BHBA theories. The glucogenic theory has been tested by measuring effects of intraruminal (Balch et al., 1967; Rook and Balch, 1961; Rook et al., 1965) intravenous (Fisher and Elliott, 1966; Rao et al., 1973; Storry and Rook, 1965) or abomasal (Spires, 1974; Vik-Mo et al., 1974a; Frobish and Davis, 1977) infusions of glucose or propionate on milk fat production. Results have been inconclusive in supporting or refuting the glucogenic theory. Frobish and

Davis (1977) observed no increase in plasma insulin concentration with abomasal propionate infusion and concluded that under normal rumen fermentation, increased propionate per se is not the cause of the low milk fat syndrome.

Ørskov (1975) introduced the concept of non-glucogenic ratio (NGR) to determine the efficiency of utilization of ruminal VFA for body and milk fat synthesis. This ratio is essentially the proportion of energy yielding nutrients from which glucose cannot be synthesized to that which can yield either glucose or glucose precursors. Results by Ørskov (1975) indicated that for an efficient utilization of energy the NGR should be below the value of 4 while if NGR is much below 3, the partition of energy will begin to suffer insofar as milk fat test will be reduced and body fat synthesis increased. However lactating dairy cows appear to tolerate a higher NGR compared to growing animals before efficiency of fat synthesis is depressed (Ørskov, 1977). This was true for experiment I (Tables 9 and 12). The low milk fat test for treatments in experiment II (Table 19) would be expected from the low acetate to propionate ratios (Table 22).

Armstrong and Blaxter (1965) with lactating goats and Ørskov et al., (1969) with lactating cows, showed that when milk fat test is depressed, there is a concomitant increase in the energy stored as fat in the body. This was evidenced by body weight gains obtained in experiment II (Table 21) despite lower N intakes than the estimated NRC (1978) requirements (Table 20). Ruminal infusion of acetate stimulated

milk fat secretion while propionate infusion increased body fat synthesis (Ørskov et al., 1969). The biochemical and hormonal factors involved in the control of the partition of energy between milk fat and body fat synthesis are not fully understood, but an increase in glucose or propionate concentration in peripheral blood appears to cause the adipose tissue to change from a situation of lipid mobilization to one of active synthesis (Orskov, 1977).

Plasma Free Amino Acids: In experiment II, no significant ($P > .05$) differences were noted among treatments for all the analyzed plasma free amino acids except tyrosine and leucine (Table 23). Feeding encapsulated methionine to supply approximately 15g methionine/day (Table 18) had little effect on the plasma free methionine concentration.

Plasma amino acids are markedly influenced by the level of feed intake, frequency of feeding, time of sampling and production level (Munro, 1964). Since these factors were not all controlled, especially when ad lib. feeding was practised, in order to reduce animal variation, the molar ratios of Met/Val were used to determine the treatment effect on plasma methionine concentration. It was assumed that fluctuation of valine concentration would reflect variation of the plasma essential amino acids in general (Broderick et al., 1970). Supplementation with encapsulated methionine did not significantly ($P > .05$) increase the Met/Val ratio in the present studies (Table 23). Williams et al., (1970)

reported no significant effect on Met/Val ratio when 12g of methionine in the form of encapsulated methionine was fed to lactating cows. Broderick et al., (1970) reported a significant ($P > .05$) increase in Met/Val ratio in cows fed 15g methionine in the form of encapsulated methionine compared to control. D-L-methionine fed orally (2.7g/day) failed to increase the Met/Val ratio in lambs, but with abomasal infusion, the Met/Val ratio was increased (Papas et al., 1974). Linton et al., (1968) reported elavated plasma free methionine and Met/Val ratio in steers fed encapsulated DL-methionine. Similar results were reported by Mowat and Deelstra (1972) with lambs.

Supplementation with methionine would supply the methionine to correct a deficiency and cause a general decrease in circulating essential amino acids due to demands for protein synthesis (Linton et al., 1968). Methionine would then increase in plasma relative to valine and other essential amino acids. An increase in Met/Val ratio without a concurrent decrease in plasma valine concentration would suggest that methionine supplementation did not enhance protein synthesis. The Met/Val ratios (Table 23) and milk protein content (Table 19) in the present studies suggest no response to methionine supplementation. There may have been another limiting amino acid and this prevented a response large enough to be measured. Since only one level of methionine supplementation was used in this experiment, it is difficult to say whether methionine was in excess or

limiting. It is also difficult to identify the other limiting amino acid(s) as analysis of plasma free amino acids was not complete.

With the exception of a study by Fisher (1972), intravenous infusion of methionine alone has not stimulated production of milk, milk fat or milk protein (Fisher, 1969; Teichman et al., 1969; Schwab et al., 1976). Therefore it appears that methionine is probably not first limiting or that another amino acid is co-limiting with methionine for protein synthesis in the mammary gland. Reported response from the feeding of methionine hydroxy analog (Griel et al., 1968; Holter et al., 1972; Polan et al., 1970a; Chandler et al., 1976) has been largely that of increased secretion of milk fat in early lactation and a subsequent increase in FCM rather than an increase in actual milk or secretion of milk protein.

PART TWO

Evaluation of Whole Plant Fababean Silage in Dairy Rations

INTRODUCTION

Fababean (Vicia faba L. Var. minor) has been recently introduced in Canada from Europe as a protein source in livestock rations. Fababean being a legume, has an advantage of fixing atmospheric nitrogen and could be beneficial in crop rotation. There is limited information regarding the utilization of whole plant fababean as a feed for ruminants. Recent studies by McNight and MacLeod (1977) suggest that the feeding value of fababean silage for lactating cows is comparable to that of good quality grass-legume silage. An attempt was made to study the conservation of whole plant fababean as silage and to evaluate its nutritive value compared to grass-legume silage for lactating dairy cows in the first experiment. The effect of treating the whole fababean plant, which is low in methionine, with formaldehyde before ensiling and supplementation with encapsulated methionine on milk production was studied in the second experiment.

MATERIALS AND METHODS

EXPERIMENT III

Silages: Whole fababean plants (approximately 33% DM) were field swathed when the bottom pods were black. One batch was chopped and ensiled as direct-cut in a concrete stave silo (4.9 x 15.2 m). The other batch was left in the field to wilt to approximately 37% DM. It was then chopped and ensiled in a wooden stave silo (4.3 x 14.0 m). Grass-legume

(brome grass-alfalfa, 50:50) forage (approximately 34% DM) was field chopped and ensiled in a concrete stave silo (4.9 x 15.2 m).

Feeding trial with Dairy Cows: Twelve Holstein cows in mid-lactation (approximately 4 months in lactation) were used to study the feed intake and performance of dairy cows fed fababean (direct-cut and wilted) and grass-legume silages. A switch back design (Lucas, 1956) was employed, cows being assigned to treatments and blocks at random. Cows received silage plus a concentrate mixture (Table 24) in a 40:60 ratio (DM basis) as a mixed feed for a high level of grain feeding and a 50:50 ratio for a medium level of grain feeding. In addition, cows were fed one Kg of hay (alfalfa-brome grass) daily. The various treatments were as follows:

1) Grass-legume silage plus grain mixture No. 1.
2) Direct-cut fababean silage plus grain mixture No. 2.
3) Wilted fababean silage plus grain mixture No. 2.
4) Wilted fababean silage plus grain mixture No. 3.

Treatments 1, 2 and 3 were with high grain feeding and treatment 4 was with the medium level of grain feeding. The different treatments were intended to be isonitrogenous.

The animals were housed in a stanchion barn and fed individually twice daily. Feed and orts were weighed daily and feed intake was recorded. The cows had direct access to water through automatic watering bowls. The cows were weighed at the beginning and end of each 28-day period.

Table 24. Ingredient and Chemical Composition of Concentrate Mixtures and Hay Fed to Dairy Cows (Expt. III)

	Concentrate Mixture*			Hay
	#1	#2	#3	
Ingredients	——% air dry basis——			
Rolled barley	62.0	72.3	72.0	-
Soybean meal	10.0	-	-	-
Rolled oats	10.0	10.0	10.0	-
Wheat bran	10.0	10.0	10.0	-
Molasses	5.0	5.0	5.0	-
Calcium phosphate	1.5	1.5	1.5	-
Urea	1.0	0.7	1.0	-
Vitamin premix[1]	0.5	0.5	0.5	-
Chemical composition (% DM)				
Crude protein	20.5	17.4	17.8	17.1
Acid-detergent fibre	8.2	9.3	9.7	35.5
Gross energy (Kcal/g)	4.33	4.32	4.32	4.50
Calcium	0.37	0.41	0.40	0.92
Phosphorus	0.91	0.95	0.89	0.25

[1]2.27 Kg premix contained 2,000,000 IU Vit. A, 200,000 IU Vit. D, 5,000 IU Vit. E, 908g MgO, 114g ZnO, 40g $MnSO_4.H_2O$, 20g $CuSO_4.5H_2O$ per 454 Kg mixed feed.

*Differences in concentrate mixtures intended to result in isonitrogenous rations.

The cows were milked twice daily and daily production was recorded. Two 24h-period milk samples were taken weekly for milk composition (fat, protein and SNF) determination. Weekly silage and grain samples were taken for DM determination and composited for each period for proximate analysis. About 100g of the fresh silage was also saved to make a period composite sample for VFA, lactic acid and soluble N analyses. On the last day of each experimental period, rumen fluid samples were collected 2½h after feeding via a stomach tube for VFA and ammonia-N analyses. At the same time, caudal vein blood samples were collected for plasma urea-N analysis.

EXPERIMENT IV

Silages: Whole plant fababeans (approximately 31% DM) were field swathed when the bottom pods were black. One batch was left in the field for a day and was then chopped and ensiled as untreated silage in a concrete stave silo (4.9 x 15.2 m). The other batch was directly field chopped and ensiled after treatment with formaldehyde in a wooden stave silo (4.3 x 14.0 m). The formaldehyde solution (37%) was mixed with an equal volume of water and was then sprayed on the plant material as it was being blown into the silo at a rate equivalent to 1 Kg formaldehyde/285 Kg wet plant material (1.2% DM).

Feeding Trial with Dairy Cows: Eight Holstein cows in early lactation (6 to 10 weeks after parturition) were used to study the feed intake and performance of dairy cows fed either untreated or formaldehyde-treated fababean silage supplemented with or without encapsulated methionine (Delmar Chemicals Co. Canada). A replicated 4 x 4 Latin square design was employed. Animals and treatments were assigned to squares at random. Cows were fed silage plus a concentrate mixture (Table 25) in a 45:55 ratio (DM basis) as a complete feed. The methionine supplement was fed separately to supply approximately 13g methionine/cow/day. In addition cows received 2 Kg hay per day. The various treatments were as follows:

1) Untreated fababean silage plus concentrate mixture.

2) Untreated fababean silage plus concentrate mixture plus methionine supplement.
3) Formaldehyde-treated fababean silage plus concentrate mixture.
4) Formaldehyde-treated fababean silage plus concentrate mixture plus methionine supplement.

Experimental periods consisted of 14 days for ration adjustment and 14 days for data collection.

The animals were weighed at the beginning and end of each period. They were housed in a stanchion barn and fed individually twice daily. The cows had direct access to water through automatic watering bowls. Wood shavings were used for bedding. Feed and orts were weighed daily and the daily feed intake was recorded. Cows were milked twice daily and daily production was recorded. Two 24h-period milk samples were taken weekly for fat, protein and SNF determination. Weekly silage and grain samples were taken for DM determination and were then composited for each period for proximate analysis. In addition about 100g of fresh silage was saved to make a period composite sample for VFA, pH, lactic acid and soluble N analyses.

During the last week of each experimental period, total collection of feces and urine from cows in one square was carried out for 3 days. Urine was collected via indwelling catheters. Feed, orts, feces and urine were sampled daily and then pooled to make a sample for each cow at the end of the 3 days. Rumen fluid samples were collected via a stomach

Table 25. Ingredient and Chemical Composition of Concentrate Mixture and Hay Fed to Dairy Cows (Expt. IV)

	Concentrate Mixture	Hay
Ingredients	% air dry basis	
Rolled barley	71.5	-
Rolled oats	10.0	-
Wheat bran	10.0	-
Molasses	3.0	-
Calcium phosphate	2.0	-
Limestone	1.5	-
Urea	1.0	-
Trace mineral salt	0.5	-
Vitamin premix[1]	0.5	-
Chemical composition (% DM)		
Crude protein	15.6	18.4
Acid-detergent fibre	8.2	34.8
Gross energy (Kcal/g)	4.22	4.52
Calcium	1.29	1.32
Phosphorus	1.22	0.22

[1] 2.27 Kg premix contained 2,000,000 IU Vit. A, 200,000 IU Vit. D, 5,000 IU Vit. E, 908g MgO, 454g Sulfur, 114g ZnO, 15g MnO_2 per 454 Kg mixed feed.

tube 2½h after the morning feeding on the last day of each period. Blood samples were taken from the caudal vein, 2½h after the morning feeding for three consecutive days during the 3rd week of each period and were then pooled for each cow for plasma free amino acid and urea-N analyses.

Laboratory Procedures: The same methods of laboratory analysis were used in both experiment III and IV. Dry matter in feeds, orts and feces was determined by drying at 60°C to a constant weight in a forced air oven. Nitrogen in the oven-dried samples was determined by macro-Kjeldahl method (AOAC, 1970). Gross energy was determined on oven-dried samples with an adiabatic oxygen bomb calorimeter. ADF and ADF-insoluble N were analyzed according to Goering and Van Soest (1970) methods.

Milk samples were analyzed for butterfat with Milko-tester (AOAC, 1975), solids-not-fat by Golding beads (Golding, 1959) and protein by acid orange G dye binding method (Ashworth et al., 1960).

Fresh silage samples were processed for N solubility and VFA determination as described previously for experiments I and II. Nitrogen solubility was determined by shaking a sample of dry feed containing 50 mg of nitrogen in 200 ml of 0.15M NaCl solution for 1h at 39°C, after adjusting the pH to 6.5 (Crooker et al., 1978). Volatile fatty acids in rumen fluid and silage samples were determined by gas-liquid chromatograph according to the method of Erwin et al., (1961). Lactic acid in silage samples was

analyzed according to Baker and Summerson (1941). Ammonia-N in the rumen fluid and silage samples was determined with an ammonia electrode (Orion Model 95 - 10). Blood plasma urea-N was analyzed with an autoanalyzer (Marsh et al., 1965). Amino acid analysis of feed samples and blood plasma samples for experiment IV was carried out as previously outlined for experiment II.

Statistical Analysis: All data in experiment III were statistically analyzed according to Lucas (1956) except for VFA data which was analyzed in a one way classification because of several missing values. The data in experiment IV were analyzed as a Latin square design and the means were subjected to the Student-Newman-Keul (SNK) test (Snedecor and Cochran, 1967).

RESULTS

EXPERIMENT III

Silage Chemical Composition: The fababean silages had substantially higher N contents than the grass-legume (GL) silage (Table 26). The proportion of total N that was soluble in either water or .15M NaCl solution was higher in the GL silage compared to the fababean silages. Wilting the fababean plant material before ensiling slightly increased N solubility in the resulting silage. Total organic acid content was somewhat similar for GL and wilted fababean (WFB) silages but slightly lower for direct-cut fababean (FB) silage. The GL silage had slightly lower pH than the fababean silages. All silages had high ADIN in the dry matter.

Feed Consumption by Dairy Cows: Average daily silage DM consumption was greater ($P < .05$) for cows fed the FB silage compared to those fed the GL silage (Table 27). Wilting did not affect ($P > .05$) silage DM consumption with the same level of grain feeding. The medium level of grain feeding led to a significantly ($P < .01$) higher silage DM consumption compared to the high level of grain feeding. Total DM consumption was not different ($P > .05$) among treatments, but was slightly higher for cows fed WFB silage with the medium level of grain in the diet. Average DM consumption per unit body weight followed the same trend as total DM consumption. The forage to concentrate ratio was somewhat different from

Table 26. Chemical Composition of Experimental Silages Fed to Dairy Cows (Expt. III)

Items	Silages		
	Grass-legume	Direct-cut Fababeans	Wilted Fababeans
Dry matter content, %	35	33	37
	% DM		
Crude protein	12.9	20.1	20.3
Acid-detergent-insoluble N	0.72	0.89	1.15
Acid-detergent fibre	44.7	36.9	37.5
Gross energy (Kcal/g)	4.599	4.476	4.342
Calcium	0.97	0.80	1.00
Phosphorus	0.25	0.25	0.25
Total organic acids	5.48	4.61	5.02
Lactic acid	3.07	1.64	2.39
Acetic acid	1.73	2.25	1.97
Propionic acid	0.16	0.27	0.30
Butyric acid	0.52	0.45	0.36
pH	4.50	4.60	4.60
Percent of total N			
Water-soluble N	60.4	31.9	37.8
0.15M NaCl-soluble N	52.4	33.5	36.4
Ammonia-N	2.7	2.8	2.8
Acid-detergent insoluble N	34.9	27.7	35.4

Table 27. Average Daily Dry Matter and Nutrient Intake of Dairy Cows Fed Experimental Diets (Expt. III)

Items	Treatments: Silage	Grass-legume	Fababeans: Direct-cut	Fababeans: Wilted	Fababeans: Wilted	SE±
	Grain Level	High	High	High	Medium	
Dry matter intake (Kg/day)						
Silage		6.6^{Aa}	7.7^{Ab}	7.0^{Aab}	10.0^{Bc}	0.3
Concentrate		10.2	9.0	10.0	8.3	0.5
Hay		0.9	0.9	0.9	0.9	-
Total		17.7	17.6	17.9	19.2	0.9
Dry matter intake (% Body weight)		3.1	2.9	2.9	3.3	0.3
Silage (% of total DMI)		37.3	43.7	39.1	52.1	-
Forage:concentrate		42:58	49:51	44:56	57:43	-
Composition of total ration						
Crude protein, %		17.5	17.7	18.6	19.1	-
Acid-detergent fibre, %		23.2	22.7	21.7	25.5	-
Gross energy (Mcal/Kg DMI)		4.44	4.40	4.34	4.34	-

^{A,B}Means with different superscripts are significantly different ($P < .01$).

^{a,b,c}Means with different superscripts are significantly different ($P < .05$).

the 40:60 and 50:50 (high grain and medium grain respectively) originally set at the beginning of the experiment. Percentage CP and ADF were somewhat similar for the high grain diets but slightly higher for the medium grain diet.

Milk Yields and Composition: Mean daily milk yield was not different ($P > .05$) among treatments (Table 28). Milk fat content was not different ($P > .05$) among treatments but tended to be slightly higher for cows fed the GL silage and the WFB silage-medium grain diets. Fat-corrected milk yield and yield of milk protein and fat were not different ($P > .05$) among treatments. Milk protein, solids-not-fat and total solids contents were similar among treatments. The level of milk production and milk composition were not affected ($P > .05$) by reducing the amount of grain fed.

Ruminal VFA, ammonia-N and Plasma urea-N: Total ruminal VFA concentration was not different among treatments (Table 29). The molar percentages of acetate, butyrate and isobutyrate were similar among treatments. Cows fed the FB silage-high grain diet had higher ($P < .05$) molar proportions of propionate than cows fed the rest of the diets. Cows fed the WFB silage-containing diets had higher ($P < .05$) molar proportions of valerate and isovalerate than those fed the rest of the diets. The acetate to propionate and non-glucogenic ratios were lower ($P < .05$) for cows fed the FB silage-high grain diet than those fed either of the other diets. Rumen ammonia-N and blood plasma urea-N concentrations were not

different ($P > .05$) among treatments, but were slightly higher for cows fed the GL silage diet.

Table 28. Average Daily Milk Production and Milk Composition of Dairy Cows Fed Experimental Diets (Expt. III)

Items	Treatments				
	Silage	Grass-legume	Fababeans		
			Direct-cut	Wilted	Wilted
	Grain Level	High	High	High	Medium	SE±
Milk yield (Kg/day)		22.9	22.4	22.0	23.5	1.4
Butterfat, %		3.67	3.51	3.44	3.62	0.16
4% Fat-corrected milk (Kg/day)		21.7	20.7	21.0	22.2	1.2
Protein, %		3.50	3.45	3.42	3.42	0.08
Solids-not-fat, %		8.55	8.59	8.34	8.51	0.13
Total solids, %		12.22	12.10	11.78	12.13	0.12
Butterfat yield (Kg/day)		0.84	0.79	0.76	0.85	0.04
Protein yield (Kg/day)		0.80	0.77	0.75	0.81	0.08

Table 29. Rumen Volatile Fatty Acid Concentration and Molar Ratios, Ammonia-N and Blood Plasma Urea-N Levels of Dairy Cows Fed Experimental Diets (Expt. III)

	Treatments				
	Silage	Grass-legume	Fababeans		
			Direct-cut	Wilted	Wilted
Items	Grain Level	High	High	High	Medium	SE±
Rumen ammonia-N (mg/100ml)		16.8	10.3	12.0	12.3	2.1
Plasma urea-N (mg/100ml)		23.3	20.3	20.0	22.7	1.6
Total rumen VFA (mM/l)		73.4	72.7	76.2	78.4	1.9
Individual VFA's (Molar %)						
Acetic		66.31	62.65	66.50	67.23	1.2
Propionic		17.92[a]	25.63[b]	18.71[a]	18.07[a]	0.80
Butyric		12.69	9.23	11.35	10.97	0.80
Isobutyric		0.83	0.51	0.82	0.87	0.09
Valeric		1.15[a]	1.08[a]	1.29[b]	1.37[b]	0.06
Isovaleric		1.10[a]	0.90[a]	1.33[b]	1.49[b]	0.12
Acetate:propionate		3.70[a]	2.44[b]	3.55[a]	3.72[a]	0.31
Non-glucogenic ratio[1]		4.87[a]	3.08[b]	4.52[a]	4.66[a]	0.34

[1]Non-glucogenic ratio = (Acetate + 2 Butyrate + Valerate)/(Propionate + Valerate); (Ørskov, 1977).

[a,b]Means with different superscripts are significantly different ($P < .05$).

EXPERIMENT IV

Silage Chemical Composition: The formaldehyde-treated fababean (FFB) silage contained less DM than the untreated fababean (FB) silage (Table 30). Crude protein percentage was slightly higher in the FFB silage than the FB silage. There was some heat damage in both silages as indicated by the high ADIN in the dry matter (Goering et al., 1972). Formaldehyde treatment slightly decreased the degree of proteolysis during ensilage. This is indicated by the lower portions of total N in soluble and ammonia forms in the FFB silage compared to the FB silage. Formaldehyde, apparently, did not affect silage fermentation as both silages had somewhat similar quantities of organic acids and pH values.

Total amino acid and individual amino acid contents of the FB and FFB silages were relatively similar (Table 31). The sulfur-amino acids levels, particularly methionine, were relatively low in the fababean silage compared to the hay or the concentrate mixture.

Feed Consumption by Dairy Cows: Average daily silage DM consumption was not different ($P > .05$) among treatments, but was slightly lower for cows fed the FFB silage (Table 32). Total DM consumption was not different ($P > .05$) among treatments, nor was DM consumption per unit body weight. Supplementing the diets with methionine did not affect either silage DM or total DM consumption. Crude protein

Table 30. Chemical Composition of Experimental Silages Fed to Dairy Cows (Expt. IV)

	Silages	
	Untreated Fababean	Formaldehyde-treated fababean
Dry matter content, %	33.0	30.5
	% DM	
Crude protein	18.0	19.1
Acid-detergent insoluble N	0.71	0.70
Acid-detergent fibre	38.2	37.8
Gross energy (Kcal/g)	4.40	4.42
Calcium	0.53	0.56
Phosphorus	0.33	0.34
Total organic acids	5.51	5.31
Lactic acid	2.12	1.90
Acetic acid	2.51	2.90
Propionic acid	0.34	0.37
Butyric acid	0.54	0.14
pH	4.80	4.70
% total N		
Water-soluble N	42.0	37.3
0.15M NaCl-soluble N	33.6	31.3
Ammonia-N	12.0	9.6
Acid-detergent insoluble N	24.7	22.9

Table 31. Amino Acid Content of Experimental Diets Fed to Dairy Cows (Expt. IV)[1]

Amino Acid	Silage		Concentrate mixture	Hay
	Untreated	Formaldehyde-treated Fababean		
	g/100g total aa			
Lysine	5.81	5.64	3.57	5.89
Histidine	2.03	1.99	1.91	1.96
Arginine	5.56	5.47	5.48	4.99
Aspartic acid	11.09	11.72	8.73	14.86
Threonine	4.02	4.19	3.72	5.59
Serine	4.16	4.21	4.13	4.64
Glutamic acid	16.21	18.51	25.81	9.45
Proline	5.32	6.54	9.09	7.22
Glycine	5.18	4.23	4.64	5.70
Alanine	7.31	5.35	4.71	6.44
Cystine	1.35	1.19	2.35	1.45
Valine	6.29	5.12	5.89	7.02
Methionine	0.80	0.87	1.32	1.70
Isoleucine	5.14	5.20	4.13	5.26
Leucine	8.60	8.82	7.82	9.11
Tyrosine	1.51	1.69	1.41	1.89
Phenylalanine	9.63	9.62	5.30	6.83
Total aa (% DM)	13.8	14.2	12.2	16.1

[1]Values not statistically analyzed and represent analysis on composite samples.

Table 32. Average Daily Dry Matter and Nutrient Intake of Dairy Cows Fed Experimental Diets (Expt. IV)

Items	Treatments: Fababean Silage	Untreated		Formaldehyde-treated		SE±
	Encapsulated methionine	-	+	-	+	
Dry matter intake, (KG/day)						
Silage		8.7	8.8	8.0	8.3	0.2
Concentrate		10.8	10.8	11.1	11.2	0.3
Hay		1.8	1.8	1.8	1.8	-
Total		21.3	21.4	20.9	21.3	0.4
Dry matter intake, (% body weight)		3.53	3.54	3.47	3.52	0.08
Silage (% of total DMI)		40.8	41.1	38.3	39.0	-
Forage:concentrate		49:51	50:50	47:53	47:53	-
Composition of total ration						
Crude protein, %		16.8	16.8	17.2	17.2	-
Soluble N (% of N intake)		34.3	34.3	33.4	33.4	-
Acid-detergent fibre, %		21.1	21.2	20.8	20.4	-
Gross energy (Mcal/Kg DMI/day)		4.27	4.13	4.00	4.14	-

and ADF levels in the diets were similar for all diets.

Animals fed encapsulated methionine-supplemented diets consumed approximately 13g Met/day more than those on unsupplemented diets (Table 33). The consumption of sulfur containing amino acids (Met + Cys) was slightly higher from the methionine-supplemented diets compared to unsupplemented diets. The consumption of other essential amino acids was relatively similar among treatments.

Milk Yields and Composition: Average daily milk and FCM yields were not different ($P > .05$) among treatments (Table 34). Milk fat, protein, solids-not-fat and total solids contents were not different ($P > .05$) among treatments. Methionine supplementation had no significant ($P > .05$) effect on milk yields and milk composition. Milk protein and fat yields and milk persistencies were not different ($P > .05$) among treatments.

Digestibility and Nitrogen Balance Trial: Apparent digestibilities of DM and CP were not different ($P > .05$) among treatments (Table 35). However cows fed the FB silage without methionine supplementation had slightly higher digestibilities of DM and CP than cows fed either of the other diets. Apparent digestibilities of ADF and energy were higher ($P < .05$) for cows fed the FB silage without methionine supplementation than cows fed the rest of the diets. Formaldehyde treatment slightly decreased the CP digestibility in the diet without methionine supplementation.

Table 33. Average Daily Intake of Sulfur-Containing Amino Acids and Other Essential Amino Acids of Dairy Cows Fed Experimental Diets (Expt. IV)[1]

Fababean silage	Untreated		Formaldehyde-treated	
Encapsulated methionine	-	+	-	+
Amino Acid	g/day			
Cystine	48.8	50.0	47.0	47.8
Methionine	30.2	43.4	31.1	44.5
Total S-aa	79.0	93.4	78.1	92.3
Arginine	145.5	146.3	143.1	145.9
Histidine	52.4	52.6	44.5	45.9
Threonine	107.7	108.2	108.3	110.5
Isoleucine	124.6	125.2	123.5	126.0
Leucine	220.8	221.9	220.6	225.0
Lysine	127.0	127.8	122.9	125.6
Phenylalanine	194.7	196.0	190.5	195.0
Valine	164.5	165.6	149.3	152.8

[1]Values are based on single analysis of composite feed samples and average feed consumption and are not statistically analyzed.

Table 34. Average Daily Milk Yields and Milk Composition of Dairy Cows Fed Experimental Diets (Expt. IV)

Items	Treatments: Fababean silage	Untreated		Formaldehyde-treated		SE±
	Encapsulated methionine	-	+	-	+	
Milk yield (Kg/day)		24.2	23.8	23.5	24.4	0.5
Butterfat, %		3.79	3.78	3.75	3.79	0.07
4% Fat-corrected milk (Kg/day)		23.4	22.9	22.6	23.6	0.4
Protein, %		3.42	3.47	3.46	3.46	0.04
Solids-not-fat, %		8.48	8.53	8.47	8.43	0.04
Total solids, %		12.27	12.31	12.22	12.22	0.08
Butterfat yield (Kg/day)		0.91	0.89	0.90	0.92	0.02
Protein yield (Kg/day)		0.82	0.83	0.81	0.84	0.02
Persistency[1], %		79.9	79.0	78.8	80.2	1.2

[1]Persistency = 100 x (Treatment milk yield/Pre-treatment milk yield).

Table 35. Apparent Digestibility Coefficients and Digestible Nutrient Intake of Dairy Cows Fed Experimental Diets (Expt. IV)

Items	Treatments: Fababean silage	Untreated		Formaldehyde-treated		SE±
	Encapsulated methionine	-	+	-	+	
Apparent digestibility coefficient (%)						
Dry matter		68.3	62.9	62.6	63.0	1.4
Crude protein		69.5	65.0	64.4	64.1	1.9
Acid-detergent fibre[1]		42.6[a]	34.2[b]	30.2[b]	30.0[b]	2.2
Gross energy[1]		67.4[a]	61.5[b]	61.0[b]	61.9[b]	1.4
Digestible nutrient intake (Kg/day)						
Crude protein		2.62	2.39	2.26	2.33	0.10
Acid-detergent fibre		1.98	1.73	1.05	1.42	0.23
Energy (Mcal/Kg DMI/day)		2.88	2.54	2.44	2.56	0.11
Crude protein intake/NRC requirements[2] (%)		147.9	146.9	143.3	141.6	-
DE intake/NRC requirements (%)		116.5	104.3	98.7	103.0	-

[a,b]Means with different superscripts are significantly different ($P < .05$).

[1]Orthogonal contrasts showed a significant ($P < .05$) effect of formaldehyde treatment.

[2]Protein and DE requirements for maintenance plus milk production estimated from NRC (1978) Tables.

Orthogonal contrasts revealed a significant ($P < .05$) depressing effect of formaldehyde treatment on ADF and energy digestibilities. Although methionine supplementation depressed ($P < .05$) the apparent digestibilities of ADF and energy in cows receiving the FB silage diet, the overall effect on these two parameters was not significant ($P > .05$).

Average digestible CP, ADF and energy intake were not different ($P > .05$) among treatments but were slightly higher for cows fed FB silage without methionine supplementation.

Average daily N intake was not different among treatments (Table 36). Methionine supplementation slightly increased N intake only in cows fed the FFB silage. Fecal and urinal N losses were not different ($P > .05$) among treatments; however cows fed the FFB silage without methionine supplementation excreted slightly more N in urine than cows fed the rest of the diets. The mean daily quantity of N absorbed and N excreted in milk were not different ($P > .05$) among treatments. Average daily N retained was positive for all cows except those fed the FFB silage without methionine supplementation, but the difference was not significant ($P > .05$).

There were no significant ($P > .05$) differences among treatments when N losses were expressed as percentages of daily intake. However cows fed the FFB silage without methionine supplementation lost a somewhat larger proportion of their N intake in urine. This led to less productive N

Table 36. Average Daily Nitrogen Intake and Utilization and Body Weight Changes of Dairy Cows Fed Experimental Diets (Expt. IV)

Items	Treatments: Fababean Silage	Untreated		Formaldehyde-treated		SE±
	Encapsulated methionine	-	+	-	+	
Nitrogen balance, g/day						
Ingested		603	590	562	581	18
Soluble N		207	204	189	193	2
Insoluble N		396	386	373	388	5
Fecal		184	208	201	208	10
Urine		227	243	275*	228	16
Milk		126	129	128	131	3
Absorbed (apparent)		419	382	361	373	17
Retained[1]		66	10	-42	14	26
Productive[2]		192	139	86	145	24
Percent of N intake						
Soluble N		34.3	34.6	33.6	33.2	0.2
Fecal		30.5	35.0	35.6	35.9	1.9
Urine		37.5	41.4	48.7	39.3	3.1
Milk		21.2	21.9	22.8	22.5	0.6
Absorbed (apparent)		69.5	65.0	64.4	64.1	1.9
Retained		10.9	1.7	-7.5	2.3	4.4
Productive		32.0	23.6	15.3	24.8	3.6

CONTINUED .....

Table 36. CONTINUED

Items	Treatments: Fababean Silage	Untreated		Formaldehyde-treated		SE±
	Encapsulated methionine	-	+	-	+	
Percent of Apparently Absorbed N						
Urine		54.2	63.6	75.1	62.9	5.3
Milk		30.4	33.8	35.4	35.1	2.0
Retained		15.4	2.6	-11.5	3.8	5.5
Productive		45.8	36.4	23.9	38.9	6.8
Body Weight gain, Kg/day		0.35	0.69	0.34	0.27	0.15

[1]Retained N = Ingested N - (Fecal N + Urine N + Milk N).

[2]Productive N = Retained N + Milk N.

*High value due to high losses from one cow in 3rd experimental period (Appendix II, Table 5).

and retained N per unit of intake. The efficiency of conversion of absorbed N into milk N was similar among treatments. Daily body weight gains were not different ($P > .05$) among treatments (Table 36).

Ruminal VFA, ammonia-N and Plasma Urea-N: Total ruminal VFA concentration was not different ($P > .05$) among treatments, but was slightly higher in cows fed the FB silage (Table 37). The molar proportions of the individual VFA were similar among treatments. Neither formaldehyde treatment nor methionine supplementation had any significant ($P > .05$) effect on the molar proportions of the individual VFA. The acetate to propionate ratio was not different ($P > .05$) among treatments. However the non-glucogenic ratio (NGR) for cows fed the FB silage with methionine supplementation was higher ($P < .05$) than that for cows fed the FFB silage with methionine supplementation. Ruminal ammonia-N and blood plasma urea-N concentrations were not different ($P > .05$) among treatments.

Plasma Amino Acids: Plasma amino acid concentrations were not different ($P > .05$) among treatments (Table 38). Feeding the FFB silage did not significantly ($P > .05$) affect plasma amino acids concentrations compared to the FB silage. Supplementation with encapsulated methionine had no significant ($P > .05$) effect on either plasma methionine levels or Met/Val ratio.

Table 37. Rumen Volatile Fatty Acid Concentration and Molar Ratios, Ammonia-Nitrogen and Blood Plasma Urea-Nitrogen Levels of Dairy Cows Fed Experimental Diets (Expt. IV)

Items	Treatments: Fababean Silage	Untreated		Formaldehyde-treated		SE±
	Encapsulated methionine	-	+	-	+	
Rumen ammonia-N (mg/100ml)		10.6	12.8	9.8	10.1	1.3
Plasma urea-N (mg/100ml)		24.5	25.2	23.5	24.1	0.9
Total ruminal VFA (mM/l)		72.0	68.3	77.3	80.4	7.9
Individual VFA (molar %)						
Acetic		62.60	63.87	62.27	61.24	0.79
Propionic		22.16	21.05	24.25	25.43	1.74
Butyric		11.47	11.50	10.15	10.38	0.61
Isobutyric		1.02	0.95	0.86	0.77	0.10
Valeric		1.30	0.98	1.28	1.12	0.10
Isovaleric		1.45	1.65	1.19	1.06	0.22
Acetate:propionate		2.9	3.1	2.7	2.6	0.2
Non-glucogenic ratio[1]		3.9^{ab}	4.1^{a}	3.4^{ab}	3.3^{b}	0.2

[1]Non-glucogenic ratio = (Acetate + 2 Butyrate + Valerate)/(Propionate + Valerate); (Ørskov, 1977).

[a,b]Means with different superscripts are significantly different ($P < .05$).

Table 38. Plasma Free Amino Acid Concentration of Cows Fed Experimental Diets (Expt. IV)*

Amino Acid	Treatments: Fababean silage	Untreated		Formaldehyde-treated		SE±
	Encapsulated methionine	-	+	-	+	
		µmole/100ml				
Serine		7.67	6.18	5.03	6.45	0.50
Proline		6.02	5.32	4.28	7.36	0.94
Glycine		21.62	20.00	20.52	27.08	2.76
Alanine		18.09	19.61	18.88	16.78	2.47
Citrulline		5.42	6.08	5.22	6.02	0.50
Tyrosine		4.14	3.72	3.25	3.26	0.26
Threonine		4.18	5.00	6.54	6.36	0.70
Isoleucine		11.19	9.71	9.61	8.04	0.86
Leucine		12.90	10.54	11.50	10.31	0.93
Methionine		1.34	1.48	1.42	1.39	0.18
Valine		23.49	22.19	27.42	20.68	1.90
Met/Val		0.056	0.069	0.056	0.069	.008

*Amino acid analysis performed only on the long column.

DISCUSSION

Silage Chemical Composition: In experiment III the grass-legume (GL) silage underwent more extensive fermentation compared to either direct-cut fababean (FB) or wilted fababean (WFB) silage as indicated by the slightly higher total organic acid content in the GL silage than the FB or WFB silages (Table 26). Prewilting the fababean before ensilage did not restrict fermentation as might be expected. McDonald and Edwards (1976) concluded that wilting conserves water-soluble carbohydrates (WSC) and tends to limit amino acid breakdown by clostridia but does not completely inhibit proteolysis.

The silages contained somewhat similar contents of ammonia-N but N solubility was higher in the GL silage than the fababean silages. Wilting tended to increase the water-soluble N fraction of the silage (Table 26). Kemble and Macpherson (1954) reported a 20% breakdown of protein to amino acids by wilting perennial ryegrass for three days. Brady (1960) reported a marked increase in NPN content of grass and leguminous fodder under conditions of slow wilting. Although low DM content-silages have higher DM losses during fermentation as well as loss of nutrients (McDonald et al., 1968), wilting can be either useful or of little value depending upon length of exposure, the effects on available carbohydrates and the influence of other factors on fermentation (Weise and Kuntzel, 1975; Marsh, 1979).

In experiment IV (Table 30), formaldehyde treatment

(1.2% DM) had little effect on total organic acid content of the silage in contrast to previous results (Barry and Fennessy, 1972; Valentine and Brown, 1973). Waldo (1977) in summarizing data of previous experiments, concluded that formaldehyde treatment decreased total organic acid content by 23%. Lactic acid content was slightly lower in the formaldehyde-treated (FFB) silage compared to untreated (FB) silage as expected (Waldo, 1977). The FFB silage contained slightly higher acetic acid content than the FB silage. A slight increase in acetic acid content by formaldehyde treatment has been reported in some experiments (Waldo et al., 1975; Valentine and Radcliffe, 1975; Barry, 1976a) but others have reported reduced acetate content with formaldehyde treatment (Barry, 1975; Barry and Fennessy, 1972; Honig and Rohr, 1973). The FFB silage had low butyric acid content compared to the FB silage in agreement with previous experiments in which formaldehyde decreased butyric acid content by 54% (Waldo, 1977).

Both the FFB and FB silages had higher pH values than those for corn silages of comparable DM (Table 30). Previous work (Valentine and Brown, 1973; Waldo, 1977) recorded higher pH values for formaldehyde-treated compared to untreated silages indicating that formaldehyde acts as a sterilant reducing bacterial fermentation. However, Valentine and Radcliffe (1975) reported pH values of 4.3 and 4.4 respectively for 0.6% and 1.2% (% DM) formaldehyde-treated silage compared to a pH of 5.0 for untreated silage. The high pH values obtained in the present studies could be due to increased

buffering capacity (Woolford, 1972) or may be an indication of secondary fermentation caused by degradation of formaldehyde to CO_2 during ensiling (Pederson et al., 1973; Honig and Rohr, 1973).

Formaldehyde treatment slightly reduced N solubility (Table 30). In addition, treatment with formaldehyde decreased ammonia-N content of the silage as previously reported in many studies (Waldo, 1977). The percentage of total N that was in the form of acid-detergent insoluble N (ADIN) was high for silages in both experiments III and IV (Tables 26 and 30) and is indicative of heat damage in the silages (Goering et al., 1972; Yu and Thomas, 1975; Yu and Veira, 1977). There is no general agreement on defining the ADIN concentration in dry matter at which heat damage starts. Georing et al., (1974) used 0.29%; Thomas et al., (1972) used 0.36% and Rook et al., (1974) used 0.40% but in all cases extent of heating was positively related to ADIN (% DM or % total N). The data in the present studies (0.7 to 1.15%; Tables 26 and 30) are well above these figures. The extent of heating per se is also a function of temperature and time (Yu and Thomas, 1975). Wilting tended to increase the ADIN content and therefore heat damage in experiment III (Table 30), in agreement with Yu (1976) who showed that haylage (50% DM) is more susceptible to heat damage (browning) than direct-cut silage. Reduced N utilization in animals fed haylage compared to low DM silage has been directly related to heat damage and therefore ADIN (Yu and Thomas, 1975; Thomas et al., 1972; Goering et al., 1973).

The FFB and FB silages in experiment IV had relatively similar total amino-N content and individual amino acids (Table 31). This is surprising since Whittenbury et al., (1967) reported a breakdown of 50 to 60% of the protein in crops ensiled directly and Barry et al., (1973) showed a marked reduction in proteolysis with the addition of formaldehyde prior to ensiling. However, Beever et al., (1977) reported relatively similar total amino-N content for formaldehyde treated (6g/100g CP) and untreated perennial ryegrass silage. Fababeans contain a very low level of sulfur amino acids, particularly methionine compared to soybean meal or barley (Blair, 1978). The methionine level in fababean silage was higher (Table 31) than that reported for the grain (Marquadt and Campbell, 1974) but is still relatively low compared to that in the hay or concentrate mixture.

Feed Consumption by Dairy Cows: In experiment III, cows fed the FB silage consumed higher ($P < .05$) silage DM than those fed the GL silage (Table 27). McKnight and MacLeod (1977) reported higher ($P < .05$) DM intakes of cows fed fababean silage compared to those fed grass-legume silage. The lower intake of GL silage compared to FB silage could have been due to the slightly higher total organic acid content in the GL silage (McLeod et al., 1970). The slightly higher acetic acid content in the FB silage compared to the GL silage (Table 26) did not affect its intake. Hutchinson and Wilkins (1971) concluded that high acetate per se is

unlikely to result in reduced intake of silage.

Wilting the fababean silage did not improve intake (Table 27) contrary to previous reports (Harris and Raymond, 1963; Jackson and Forbes, 1970; Hinks et al., 1976). Fermentation in the WFB silage was not restricted as normally occurs in wilted forages (MacDonald and Edwards, 1976; Marsh, 1979). Therefore the higher total organic acid content in the WFB compared to the FB silage (Table 26) could account for its lower intake (McLeod et al., 1970). Dry matter consumption of the WFB increased significantly ($P < .01$) with the medium level of grain feeding. This might be expected with the medium compared to the high level of grain supplement, since supplementation with cereal diets can cause substantial reduction in rate of cellulose digestion and therefore in voluntary intake of roughage (Ørskov and Fraser, 1975).

No significant ($P > .05$) differences were observed among the treatments of experiment IV for silage DM consumption (Table 32). Valentine and Radcliffe (1975) reported higher ($P < .01$ and $P < .05$, respectively) silage DM consumption by dairy cows fed 0.6% and 1.2% (DM) formadehyde-treated grass-clover silage compared to untreated silage. The rate of formaldehyde application in the present studies was about 1.2% DM. Although formaldehyde preparation of silage inhibits in silo fermentation and partly protects protein from rumen degradation (Barry et al., 1973; Valentine and Brown, 1973), its effects on intake appear to be related to levels of application used. When formaldehyde was applied

at 3.2 to 6.4% of DM, DM intake of lucerne silage by sheep was reduced (Brown and Valentine, 1972) though performance in terms of wool growth was not adversely affected. At lower rates of application (0.9% DM), DM intake and wool growth was not affected but digestibility of protein and DM increased. Barry (1975) reported improved DM intakes of formaldehyde-treated silages fed to sheep where the application rate was 0.55% DM. Rates of application in excess of 8g formaldehyde per 100g CP were shown to lower DM intake (Wilkins and Cook, 1975). The optimal range of 3 to 5g/100g CP has been suggested (Wilkinson et al., 1976b; Barry, 1976a). The rate used in the present studies was approximately 6g/100g CP somewhat above the recommended range.

Feeding encapsulated methionine to supply approximately 13g Met/day did not affect DM consumption (Table 32) in agreement with previous reports (Williams et al., 1970; Broderick et al., 1970). Consumption of untreated silage by sheep was increased with intraperitoneal infusions of methionine (Barry et al., 1973; Barry, 1976b) suggesting that the availability of the sulfur-amino acids was limiting intake in sheep because of their high requirements for sulfur-amino acids for wool growth.

Ration Digestibility: Digestibility data was collected for experiment IV only. No significant ($P > .05$) differences were noted among treatments for DM and CP digestibilities (Table 35). Generally formaldehyde treatment has decreased protein digestibility (Ettala, 1975b; Barry and Fennessy,

1973; Brown and Valentine, 1972; Waldo et al., 1973a; Valentine and Brown, 1973; Wilkins et al., 1974a). However the influence of formaldehyde in the rumen was more 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 silages. The results of CP digestibility in the present studies would be expected since N solubility was somewhat similar among the treatments.

Significant ($P < .05$) differences were noted among the treatments for ADF and energy digestibilities (Table 35). Orthogonal contrasts revealed that the formaldehyde treatment significantly ($P < .05$) decreased the digestibilities of ADF and energy. Formaldehyde treatment had little effect on DM and gross energy digestibilities in sheep (Hinks and Henderson, 1977; Valentine and Brown, 1973; Barry, 1975). However, Beever et al., (1977) reported a depression of organic matter and energy digestibilities within the rumen of sheep fed formaldehyde-treated silage.

Methionine supplementation depressed ($P < .05$) the digestibilities of ADF and energy for the FB silage and not for FFB silage diets. Methionine was reported to promote cellulose fermentation in vitro (Bull et al., 1973; Salsbury et al., 1971; Salsbury and Zikakis, 1965), but was less effective than MHA in promoting dry matter and ADF digestibilities (Bull et al., 1973; Polan et al., 1970b).

Digestible nutrient (CP, ADF and energy) intakes were

not different ($P > .05$) among treatments. The daily requirements for maintenance and milk production of crude protein and digestible energy were estimated using NRC (1978) Tables (Table 35). Crude protein intake of the cows was in excess of their estimated requirements. Digestible energy intake was marginal or below the estimated requirements for all cows except those fed the FB silage without methionine diet.

Milk Yields and Composition: No significant ($P > .05$) differences were observed among the treatments of experiment III for milk yields and composition (Table 28). The level of milk production was not affected when the amount of concentrate was reduced from 56% to 43% of the total DM when fed with WFB silage. The cows apparently compensated by increased consumption of WFB silage which resulted in similar energy intake. McKnight and MacLeod (1977) reported higher milk fat test for cows fed FB silage compared to those fed GL silage even though intake of fiber as % of diet was higher for the GL silage compared to the FB silage (20.6 vs 18.7). The fat test results in the present studies do not reflect the different intake in fibre among treatments (Table 27) or the differences among the acetate to propionate ratios (Table 29).

In experiment IV, milk yields and composition were not different ($P > .05$) among treatments (Table 34). Valentine and Radcliffe (1975) reported significantly ($P < .01$ and $P < .05$ respectively) higher milk and SNF yield and significantly ($P < .01$) higher butterfat and protein yields for

cows fed 0.6% and 1.2% (DM) formaldehyde treated silage compared to those fed untreated silage. Differences in percentages of butterfat, protein and SNF in milk were not significant except for the significantly ($P < .01$) lower milk protein % of cows offered the 1.2% (DM) formaldehyde-treated silage.

Concerning the use of daily milk production to compare silage quality, Waldo (1977) concluded that daily milk production would not easily distinguish between quality of silages when fed with grain and that daily body weight gain was a more sensitive criterion than daily milk production. Concentrates have not only the direct effect of diluting silage differences but also the indirect effect of providing energy for microbial growth.

Supplementation with encapsulated methionine had little effect on milk production or synthesis of milk protein (Table 34). The feeding of dietary encapsulated methionine to cows on corn silage-based diets (Broderick et al., 1970; Williams et al., 1970) similarly failed to elicit a response in milk or milk protein yield. Teichman et al., (1969) and Fisher (1969) found no response in milk production, milk protein or secretion of milk fat when various quantities of L- or DL-methionine were infused intravenously. However, Fisher (1972) found that daily intravenous infusion of 11.2g DL-methionine increased secretion of milk protein. The observed reponse was confounded, however, since the cows receiving methionine consumed significantly ($P < .05$) more

feed than the cows receiving the saline control. Reported response from the feeding of MHA (Chandler et al., 1976; Griel et al., 1968; Holter et al., 1972; Polan et al., 1970a) has been largely that of increased secretion of milk fat in early lactation and a subsequent increase in FCM rather than an increase in actual milk or secretion of milk protein. In vitro studies showing increased rumen protozoal numbers (Patton et al., 1970), increased rumen bacterial growth rates (Gil et al., 1973a,b,c) and increased rumen total lipid synthesis (Patton et al., 1968) suggest that the influence of dietary MHA on milk fat synthesis is largely due to its effect in the rumen. The fact that inorganic sulfur produces less but similar responses in in vitro digestion (Bull et al., 1973; Gil et al., 1973b) and in vivo digestion (Bull et al., 1973) suggests that part of the bacterial stimulation by MHA may be related to sulfur.

A comparison of the amino acid content of microbial protein with milk protein (Table 39) indicates that microbial protein is probably of excellent quality for milk production. Fababean (FB or FFB) silage protein is low in methionine, threonine and lysine. The apparent sequence of limiting amino acids relative to milk protein (Table 39) indicated that methionine, threonine and lysine were limiting in that order for milk and milk protein production in the present studies. Methionine, lysine and threonine all have been mentioned as possible amino acids limiting milk production of cows fed diets of mostly corn, soybean meal and corn

Table 39. Essential Amino Acid Content of Rumen Bacterial and Protozoal Protein, Milk Protein and the Fababean Silages*

Amino acid	Bacteria[a]	Protozoa[a]	Milk[a]	Fababean Silage	Formaldehyde-treated Fababean Silage
	% of total essential aa				
Arginine	10.5	8.9	7.0	11.3 (10)[b]	11.4 (10)
Histidine	4.1	3.7	5.4	4.4 (4)	4.1 (4)
Isoleucine	11.7	10.2	9.4	10.4 (7)	10.8 (7)
Leucine	15.4	15.9	19.6	17.5 (5)	18.3 (6)
Lysine	17.0	20.4	16.2	11.8 (3)	11.7 (3)
Cystine	2.3	2.6	1.7	2.7 (8)	2.5 (8)
Methionine	5.0	3.9	4.9	1.6 (1)	1.8 (1)
Phenylalanine	9.7	11.5	10.1	19.6 (9)	20.0 (9)
Threonine	12.1	13.2	12.4	8.2 (2)	8.7 (2)
Valine	12.2	9.7	13.3	12.8 (6)	10.6 (5)

[a]Calculated from data reported by Schwab et al., (1976).

[b]Numbers in parentheses indicate the apparent sequence of limiting amino acids for milk protein synthesis.

*Analysis for tryptophan was not performed.

silage (Ahrar and Schingoethe, 1979; Clark et al., 1973; Derrig et al., 1974; Schwab et al., 1976; Vik-Mo et al., 1974a).

The absence of a response due to methionine supplementation in the present studies, could suggest probably that the level of methionine used was not enough to offset the deficiency or that either threonine or lysine was co-limiting with methionine for milk production or the amino acids were not limiting production since protein intake was above requirements.

Nitrogen Balance: No significant ($P > .05$) differences were noted among the treatments of experiment IV for N intake and N utilization (Table 36). Treatment with formaldehyde has generally resulted in increased fecal N excretion (Chalupa, 1975), but this was not the case in the present studies. Animals fed the unsupplemented FFB silage diet tended to excrete more N in urine compared to the others although intake of soluble N was similar. This led to the negative N balance for these animals (Table 36). Formaldehyde treatment had little effect on the efficiency of conversion of absorbed N into milk. This would indicate that formaldehyde treatment did not increase the quantities of total amino acids reaching the intestines for absorption as reported in previous studies (Beever et al., 1977) or that the requirement was already being met by the high CP intake (Table 35).

The high ADIN values in the silages (Table 30) could also have affected N utilization (Yu and Thomas, 1975; Goering et al., 1973). The protein in the FFB silage was

probably overprotected by the high rate of formaldehyde (6g/100g CP) used although levels of rumen ammonia-N (Table 37) show the contrary. The presence of free formaldehyde could also have had some effect on microbial activity (Beever et al., 1977). Uncoupled fermentation, during which degradation can proceed but synthesis of microbial protein is restricted (Beever et al., 1977) may have led to reduced yields of microbial protein in the rumen of cows fed the FFB silage diets, especially as VFA production by cows on these diets was not significantly ($P > .05$) affected.

Wilkinson et al., (1976b) concluded that N utilization and intake were low on diets of direct-cut, untreated silage, due to extensive protein and carbohydrate degradation in the silo. It was thought that rumen microbial protein synthesis was probably low because over half the N consumed was NPN and all the readily available energy had already been fermented to organic acids in the silo. Hence supplementation of energy and protein has been used to improve the utilization of silage (Thomson, 1968; Forbes and Irwin, 1970; Drennan, 1973). In the present studies, formaldehyde treatment did not restrict fermentation and together with lack of digestible energy (Table 35) probably led to the low N utilization on the formaldehyde treated silage diets. Increased N retention in response to supplementation of silage with energy has been reported (Thomson, 1968). Griffiths et al., (1973) suggested that the addition of energy improved the utilization of NPN in silage by

stimulating microbial synthesis which would result in an increased flow of protein into the duodenum. Excessive N intake (Table 32) could also have reduced the efficiency of energy utilization due to the energy required for urea synthesis from ammonia (Tyrrell et al., 1970).

Methionine supplementation had little effect on N utilization (Table 36). Wilkinson et al., (1976b) showed that further responses in animal production, though not necessarily intake, could be obtained from postruminal supplementation with amino acids in animals given formaldehyde-treated silages ad lib. This showed that whilst formaldehyde treatment improved animal performance, the amounts of some limiting essential amino acids absorbed were still below the levels necessary for optimum production. In the present studies, methionine was probably not limiting production or the level used was too low to show a response.

Ruminal ammonia-N, VFA and plasma urea-N: No significant ($P > .05$) differences were noted among the treatments of experiment III for rumen ammonia-N (RAN) and blood plasma urea-N (BUN) contents (Table 29). However RAN concentration tended to be slightly higher for the GL silage diets compared to the FB and WFB silage diets. This would suggest a larger part of the FB and WFB protein bypassed the rumen possibly because the GL silage had higher N solubility compared to the FB and WFB silages (Table 26).

In experiment IV, there were no significant ($P > .05$) differences among treatments for RAN and BUN concentrations

(Table 37). This is contrary to previous reports (Barry and Fennessy, 1973; Saue et al., 1972; Wilkins et al., 1974a) in which formaldehyde treatment decreased rumen ammonia concentrations. However the results are consistent with the N solubilities of the diets which were not different ($P > .05$).

No significant ($P > .05$) differences were observed among the treatments of experiment III for total ruminal VFA concentration and molar proportions of acetate, butyrate and isobutyrate (Table 29). Cows fed the FB silage diet had higher ($P < .05$) propionate than the other cows. The significantly ($P < .05$) higher molar percentages of valerate and isobutyrate for the WFB silage-medium grain diet compared to the other diets probably indicates more degradation of amino acids, particularly the branched-chain amino acids (valine, isoleucine and leucine) by bacteria in the rumen (El-Shazly, 1952). McKnight and MacLeod (1977) did not observe any difference in rumen VFA proportions for cows fed grass compared to fababean silage.

In experiment IV, no significant ($P > .05$) differences were noted among treatments for total VFA concentration and molar proportions of individual VFA (Table 37). Formaldehyde treatment has raised the acetate to propionate ratio in the rumen in previous experiments (Honig and Rohr, 1973; Barry and Fennessy, 1973; Donald and Edwards, 1977). However Beever et al., (1977) reported no influence of formaldehyde on molar proportions of individual VFA. There is evidence to suggest conversion of silage lactate to

propionate in the rumen (Honig and Rohr, 1973; Donald and Edwards, 1977) which could explain decreased propionate production with formaldehyde treatment. The FFB and FB silages contained somewhat similar contents of lactic acid which could explain the similar acetate to propionate ratios in the present studies (Table 37).

There were significant ($P < .05$) differences among the treatments of both experiments III and IV for non-glucogenic ratios (NGR) (Tables 29 and 37). However butterfat and SNF tests were not different ($P > .05$) among treatments. The efficiency of energy utilization of milk fat secretion is usually maximized with NGR of 3 to 4 (Ørskov, 1977). Honig and Rohr (1973) obtained higher fat test from cows fed formaldehyde-treated (NGR, 4.3) compared to untreated silage (NGR, 3.5). Valentine and Radcliffe (1975) reported increased yields of milk fat but no change in fat test when silages treated with formaldehyde were fed to dairy cows. However, no VFA data was given.

Blood Plasma Amino Acids: In experiment IV, no significant ($P > .05$) differences were noted among treatments for the measured plasma free non-essential and essential amino acids (Table 38). Thus formaldehyde treatment failed to increase the quantities of amino acids absorbed from the small intestines, contrary to previous reports with formaldehyde treated-silage (Beever et al., 1977) or formaldehyde treated-casein or groundnut meal (Barry, 1976a). Hemsley et al., (1970) showed a 60% increase in the uptake of non-

ammonia-N for a sun-cured herbage treated with 4g HCHO/100g CP. Although formaldehyde treatment (6g HCHO/100g CP) of perennial ryegrass silage increased total amino acid absorption, it tended to depress the overall availability of amino acids at the duodenum (Beever et al., 1977).

It is necessary to consider the reaction of formaldehyde with protein in order to resolve the above-mentioned anomalies. The reactive sites include the terminal amino groups of lysine, the guanidyl group of arginine, the hydroxy groups of threonine and serine, the sulphydryl group of cysteine, the phenol group of tyrosine, the phenyl group of phenylalanine, the indole group of tryptophan and imidazole group of histidine (Van Dooren, 1972). Most of these sites may be available in silage or air dried materials for a chemical reaction with formaldehyde. The maximal beneficial response to be achieved with formaldehyde will probably be at a lower rate of application than the rate (6g HCHO/100g CP) used in the present studies, especially on diets like fababean silage which already contain some naturally protected protein.

Feeding encapsulated methionine (13g Met/day) had little effect on either plasma methionine concentration or Met/Val ratio (Table 38). Dietary encapsulated methionine generally has increased free methionine in plasma (Broderick et al., 1970; Linton et al., 1968; Mowat and Deelstra, 1972; Sibbald et al., 1968). Dietary MHA had, however, little or no effect on methionine concentration in plasma (Griel et al., 1968; Muller and Rodriguez, 1975; Papas et al., 1974;

Whiting et al., 1972) suggesting that the effect of dietary MHA on milk fat synthesis is due to its effects in the rumen. Williams et al., (1970) reported no response in milk production or Met/Val ratio by feeding encapsulated methionine (12g Met/day). Broderick et al., (1970) reported increased Met/Val ratios in cows fed encapsulated methionine (15g Met/day) compared to control with no effect on milk production.

SUMMARY AND CONCLUSIONS

Studies were conducted to evaluate the conservation and nutritive value of NPN-treated corn silage, untreated and formaldehyde-treated whole plant fababean silage. Whole chopped corn plant material (31 to 42% DM) was conserved as untreated, urea-treated (0.5% wet basis) and Pro-Sil-treated (1.3% to 2.2% wet basis) silage. Whole chopped fababean plant material (31 to 33% DM) was conserved by ensiling directly (FB), after wilting to 37% DM (WFB) and after treating with formaldehyde (1.2% DM) (FFB). Grass-legume silage was ensiled at 35 to 38% DM.

Recoveries of added N were high for the NPN-treated silages (95 to 100%). Lactic acid and total organic acid contents appeared to be higher in the NPN-treated silages than the untreated corn or grass-legume silage. Wilting and formaldehyde treatment did not restrict silage fermentation. The grass-legume and fababean silages contained high acid-detergent insoluble N in the dry matter, indicative of heat damage.

Results from two digestibility and N balance trials with wethers indicated no significant ($P > .05$) differences among the NPN-treated silages for apparent digestibilities of DM, CP and energy and N utilization. The apparent digestibilities of DM, CP and energy were lower ($P < .05$) for the grass-legume silage compared to urea-treated or Pro-Sil-treated (2.2%) corn silages (Expt. I). The apparent digestibility of CP was lower ($P < .05$) for the untreated corn

silage compared to the urea-treated or Pro-Sil-treated (1.3%; 1.7%) corn silages (Expt. II).

Silage DM consumption was not different ($P > .05$) when the grass-legume silage (38% DM), urea-treated (31% DM) and Pro-Sil-treated (2.2%; 32% DM) corn silages were fed to lactating Holstein cows (Expt. I). Reducing the level of grain feeding from 40% to 30% of the diet with Pro-Sil-treated silage resulted in a decrease ($P < .05$) in total DM consumption. Milk and FCM yields and milk composition were not different ($P > .05$) among treatments. Apparent digestibilities of DM and energy were lower ($P < .05$) for the grass-legume silage-containing diet than for the other diets. Nitrogen consumption and N utilization were not different ($P > .05$) among treatments. Rumen ammonia-N (RAN) and total VFA concentrations were not different ($P > .05$) among treatments but blood plasma urea-N (BUN) levels were higher ($P < .05$) for cows fed the grass-legume silage-containing diet than for cows fed the Pro-Sil-treated corn silage-containing diets.

The untreated (38% DM) and Pro-Sil-treated (1.3%; 42% DM) corn silages were fed along with grain mixtures containing SBM (P), SBM + urea (U), fababeans instead of SBM supplemented with (M) or without (F) encapsulated methionine in 45:55 ratio (DM) to lactating Holstein cows (Expt. II). Diets were Pro-Sil-treated corn silage + grain P (Pro-Sil), untreated corn (C) silage + grain U (Urea), C silage + grain M (Fababean + methionine) and C silage + grain F (Fababean).

Silage DM consumption was lower ($P < .05$) for cows fed the Pro-Sil-treated corn silage than for cows fed the untreated silage. However total DM consumption was not different ($P > .05$) among treatments. Milk and FCM yields, protein and SNF contents were not different ($P > .05$) among treatments. Milk fat test was higher ($P < .05$) for cows fed the Fababean diet than for cows fed the Pro-Sil diet. The apparent digestibility of DM was lower ($P < .05$) for the fababean-containing diets than for the other diets. The apparent digestibility of energy was lower ($P < .05$) for the Fababean + methionine diet than for Urea and Fababean diets. No significant differences ($P > .05$) were noted among treatments for the apparent digestibilities of CP and ADF. Nitrogen consumption and fecal N were lower ($P > .05$) for cows fed the Pro-Sil diet than for cows fed the other diets, but N retention was not different ($P > .05$) among treatments. Total ruminal VFA, RAN and BUN concentrations were not different ($P > .05$) among treatments.

Twelve lactating Holstein cows were fed four diets containing either GL silage (35% DM), FB (33% DM) or WFB (37% DM) plus a concentrate mixture (40:60 DM) for the high level of grain feeding regime and WFB plus a concentrate mixture (50:50 DM) for the medium level of grain feeding regime (Expt. III). Consumption of the FB silage was higher ($P < .05$) than that of the GL silage. Wilting had no effect ($P > .05$) on silage DM intake and reducing the level of grain feeding from 56% to 43% of the diet caused

an increase ($P < .01$) in the WFB silage consumption. Milk yields and milk composition were not different ($P > .05$) among treatments. Total ruminal VFA, RAN and BUN levels were not different ($P > .05$) among treatments.

Diets containing either FB (31% DM) or FFB (33% DM) and a concentrate mixture (45:55 DM) supplemented with or without methionine (13g/day) were fed to lactating Holstein cows (Expt. IV). Silage DM and total DM consumption, milk yields and milk composition were not different ($P > .05$) among treatments. The apparent digestibilities of energy and ADF were decreased ($P < .05$) by formaldehyde treatment. Nitrogen consumption and N utilization were not different ($P > .05$) among treatments. No significant ($P > .05$) differences were noted among treatments for RAN, BUN and total ruminal VFA concentrations.

Supplementation with methionine (13 to 15g/day) to high fababean-containing diets (Expt. II and IV) had no effect ($P > .05$) on feed consumption, milk yield, milk composition, blood plasma free methionine levels and Met/Val ratios.

Under the conditions of the present experiments, the following conclusions seem justified:

1. The addition of Pro-Sil to corn silage was as beneficial to silage fermentation as the addition of urea.
2. Pro-Sil-treated corn silage resulted in lactating cow performance comparable to that from urea-treated corn silage.

3. Substitution of soybean meal with fababean had no adverse effect on either DM consumption or milk production of lactating dairy cows.
4. Wilting or formaldehyde treatment of whole fababean plant material before ensiling had little effect on silage fermentation, DM consumption and animal performance.
5. Methionine supplementation (13 to 15g/day) of high fababean (grain or silage)-containing diets which were low in methionine had little effect on feed consumption, milk production and blood plasma methionine levels of lactating dairy cows.

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APPENDICES

Appendix I - Table 1. Data on Some Criteria Measured on Sheep Fed Experimental Silages (Expt. I)

Periods	Treatments		
	Grass-legume silage	Urea-treated corn silage	Pro-Sil-treated corn silage
	Silage DM intake, Kg/day		
I	0.95 (4)*	0.83 (9)	1.18 (8)
II	0.97 (9)	1.19 (8)	0.92 (4)
III	1.13 (8)	0.97 (4)	0.90 (9)
	Silage DM intake, g/KgW$^{3/4}$		
I	49.7	43.0	63.1
II	63.4	79.3	58.2
III	67.7	51.9	50.0
	DM digestibility, %		
I	57.60	67.49	67.64
II	57.21	66.80	68.79
III	57.20	63.22	64.68
	CP digestibility, %		
I	63.59	67.88	68.45
II	64.82	71.37	69.94
III	65.82	68.98	67.80
	Gross energy disgestibility, %		
I	56.37	68.08	68.59
II	55.88	67.10	70.20
III	56.40	64.12	66.19

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Appendix I - Table 1. CONTINUED

Periods	Treatments		
	Grass-legume silage	Urea-treated corn silage	Pro-Sil-treated corn silage
	Nitrogen intake, g/day		
I	18.4	16.5	22.5
II	19.9	26.2	17.3
III	23.7	21.6	17.7
	Fecal N, (% intake)		
I	36.41	32.12	31.55
II	35.18	28.63	30.06
III	34.18	31.02	32.20
	Urine N (% intake)		
I	52.72	46.06	31.71
II	46.73	51.15	54.34
III	51.48	46.76	41.81
	N retained (% intake)		
I	10.87	21.82	37.34
II	18.09	20.22	15.60
III	14.34	22.22	25.99

* Numbers in brackets designate sheep No. and sequence.

Appendix I - Table 2. Replicated Latin Square Change-Over Design and Treatment Sequence (Expt. I)

Periods/ Cows No.	Duration (days)	Treatment Sequence[1] Square I 1	2	3	4	Square II 5	6	7	8
Preliminary	14								
Period I									
Adjustment	14	A	C	D	B	D	B	C	A
Comparison	14								
Period II									
Adjustment	14	B	A	C	D	B	D	A	C
Comparison	14								
Period III									
Adjustment	14	C	D	B	A	A	C	B	D
Comparison	14								
Period IV									
Adjustment	14	D	B	A	C	C	A	D	B
Comparison	14								

[1] Treatment A, Grass-legume silage + grain No. 1.
Treatment B, Urea-treated corn silage + grain No. 2.
Treatment C, Pro-Sil-treated corn silage + grain No. 2.
Treatment D, Pro-Sil-treated corn silage + grain No. 3.

Appendix I - Table 3. Data on Some Criteria Measured on Cows Fed Experimental Diets (Expt. I)

Periods/Cows No.	1	2	3	4	5	6	7	8
	Silage DM intake (Kg/day)							
I	10.74	10.77	12.35	8.84	13.64	12.99	9.24	8.93
II	10.96	13.66	13.36	11.11	12.89	10.12	13.56	8.73
III	11.29	14.07	15.19	12.30	12.69	12.92	12.54	9.18
IV	13.36	11.14	15.93	11.66	13.35	10.72	12.94	10.22
	Concentrate DM intake (Kg/day)							
I	9.58	9.36	6.58	9.54	4.84	7.10	7.80	7.70
II	7.82	8.76	5.73	5.20	6.08	2.76	6.01	4.68
III	7.54	5.31	5.61	6.66	5.61	4.04	5.61	1.77
IV	4.24	7.40	5.08	5.55	4.94	3.50	2.82	2.29
	Total DM intake (Kg/day)							
I	22.07	21.88	20.68	20.13	20.23	21.84	18.79	18.38
II	20.53	24.17	20.84	18.06	20.72	14.63	18.71	15.97
III	20.58	21.13	22.55	20.71	20.05	18.71	19.90	12.70
IV	19.35	20.29	20.71	18.96	20.04	15.97	17.51	14.26

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Appendix I - Table 3. CONTINUED

Periods/Cows No.	1	2	3	4	5	6	7	8
	Milk yield (Kg/day)							
I	28.1	30.2	26.6	29.0	24.4	24.3	23.2	20.0
II	26.2	29.1	22.5	24.8	23.0	19.7	20.7	17.2
III	24.4	27.7	20.8	22.4	20.8	18.8	20.6	14.0
IV	21.4	27.6	20.0	21.7	20.6	15.6	18.0	13.6
	Milk fat (%)							
I	3.97	3.16	3.80	3.34	4.10	3.98	3.60	3.97
II	3.84	3.56	4.22	3.86	3.46	3.89	3.82	4.14
III	3.96	3.70	4.56	4.20	4.36	3.86	4.01	4.66
IV	4.18	3.49	4.25	3.96	4.45	3.88	3.96	4.38
	Milk protein %							
I	3.48	3.24	3.50	3.13	4.01	3.64	3.50	3.68
II	3.60	3.18	3.66	3.20	4.14	3.52	3.47	3.94
III	3.84	3.21	3.76	3.24	4.08	3.64	3.64	3.89
IV	4.05	3.56	4.02	3.32	4.44	3.84	3.72	4.10

Appendix I - Table 4. Data on Digestibility and Nitrogen Balance Trial with Dairy Cows (Expt. I)

Period/ Cows No.	5	6	7	8
	DM digestibility (%)			
I	66.05	70.08	69.54	63.37
II	63.70	62.60	64.17	66.71
III	59.91	71.44	70.74	65.63
IV	67.40	54.24	71.44	67.43
	CP digestibility (%)			
I	65.56	68.70	67.39	64.65
II	63.00	61.41	66.34	65.36
III	66.77	67.77	69.52	63.89
IV	56.63	60.29	69.77	64.61
	ADF digestibility (%)			
I	46.72	51.08	47.29	42.26
II	45.13	45.85	49.41	48.35
III	45.06	54.42	54.21	49.76
IV	49.71	36.29	59.42	51.59
	Gross energy digestibility (%)			
I	65.74	69.67	69.78	61.71
II	63.40	61.72	62.61	66.38
III	59.13	69.22	71.20	65.45
IV	66.67	52.59	71.04	67.00

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Appendix I - Table 4. CONTINUED

Period/ Cows No.	5	6	7	8
	Nitrogen Intake (g/day)			
I	441.8	517.4	408.2	457.2
II	451.6	356.2	542.4	350.6
III	497.9	411.1	470.5	288.1
IV	454.9	354.6	393.3	324.2
	Fecal N (% intake)			
I	34.44	31.30	32.61	35.35
II	37.00	38.59	33.66	34.64
III	33.23	32.33	30.48	36.11
IV	43.37	39.71	30.23	35.39
	Urine N (% intake)			
I	31.12	25.89	16.52	15.30
II	37.35	48.29	28.64	44.27
III	33.94	18.96	28.57	36.72
IV	27.72	30.96	30.72	32.48
	Nitrogen balance (% intake)			
I	44.44	42.81	50.81	47.35
II	25.65	13.12	37.70	21.09
III	32.83	48.81	40.95	27.17
IV	28.91	29.33	39.05	32.13

Appendix I - Table 5. Data on Some Criteria Measured on Sheep Fed Experimental Silages (Expt. II)

Periods	Corn Silages			
	Untreated	Urea-treated	Pro-Sil-treated (1.3%)	Pro-Sil-treated (1.7%)
	Silage DM intake, Kg/day			
I	1.23 (3)*	0.82 (4)	1.25 (6)	1.05 (2)
II	1.14 (6)	1.13 (3)	1.23 (2)	1.25 (4)
III	1.26 (4)	1.22 (2)	1.56 (3)	1.29 (6)
IV	1.21 (2)	1.15 (6)	1.32 (4)	1.46 (3)
	Silage DM intake, g/Kg$W^{3/4}$			
I	66.9	42.9	61.1	57.4
II	58.1	56.8	62.5	60.2
III	55.8	59.0	72.2	56.7
IV	57.5	48.1	55.7	60.8
	DM digestibility, %			
I	73.41	72.31	72.97	74.64
II	74.80	65.92	74.55	77.94
III	67.39	71.57	70.80	76.18
IV	71.87	73.64	70.27	74.96
	CP digestibility, %			
I	57.59	69.18	59.08	72.28
II	60.74	72.84	76.67	74.93
III	49.57	75.05	56.61	69.99
IV	55.97	71.47	70.83	70.00

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Appendix I - Table 5. CONTINUED

Periods	Corn Silages			
	Untreated	Urea-treated	Pro-Sil-treated (1.3%)	Pro-Sil-treated (1.7%)
	ADF digestibility, %			
I	55.95	55.68	58.22	59.41
II	53.88	48.77	54.94	61.18
III	47.71	52.00	52.68	63.87
IV	52.51	58.40	58.62	60.40
	Gross energy digestibility, %			
I	74.14	73.49	73.21	75.57
II	75.88	67.03	75.08	78.89
III	68.26	72.53	70.87	77.04
IV	72.76	74.48	75.72	75.59
	Nitrogen intake, g/day			
I	15.5	15.9	17.5	18.6
II	13.2	24.1	22.8	24.2
III	14.2	23.0	25.6	22.8
IV	14.3	22.4	22.7	25.8
	Fecal N (% intake)			
I	42.41	30.82	40.92	27.72
II	39.26	27.16	23.33	25.07
III	50.43	24.95	43.39	30.01
IV	44.03	28.53	29.17	30.00

CONTINUED . . . .

Appendix I - Table 5. CONTINUED

Periods	Corn Silages			
	Untreated	Urea-treated	Pro-Sil-treated (1.3%)	Pro-Sil-treated (1.7%)
	Urine N (% intake)			
I	27.10	43.34	37.97	45.85
II	19.32	38.30	50.44	33.86
III	19.20	30.36	36.06	36.26
IV	21.58	51.28	36.08	36.81
	N retained (% intake)			
I	30.49	25.84	21.11	26.43
II	41.42	34.55	26.23	41.07
III	30.37	44.69	20.55	33.73
IV	34.39	20.19	34.75	33.19

*Numbers in brackets designate sheep No. and sequence.

Appendix I - Table 6. Replicated Latin Square Design and Treatment Sequence (Expt. II)

Periods/ Cows No.	Duration (days)	Treatment Sequence[1] Square I				Square II			
		9	10	11	12	13	14	15	16
Preliminary	14								
Period I									
Adjustment	14	A	C	D	B	D	B	C	A
Comparison	14								
Period II									
Adjustment	14	B	A	C	D	B	D	A	C
Comparison	14								
Period III									
Adjustment	14	C	D	B	A	A	C	B	D
Comparison	14								
Period IV									
Adjustment	14	D	B	A	C	C	A	D	B
Comparison	14								

[1] Treatment A, Pro-Sil-treated corn silage + grain P (Pro-Sil diet).
Treatment B, Untreated corn silage + grain U (Urea diet).
Treatment C, Untreated corn silage + grain M (Fababean + methionine diet).
Treatment D, Untreated corn silage + grain F (Fababean diet).

Appendix I - Table 7. Data on Some Criteria Measured on Cows Fed Experimental Diets (Expt. II)

Periods/Cows No.	9	10	11	12	13	14	15	16
	Silage DM intake (Kg/day)							
I	8.88	10.79	6.69	7.95	8.39	6.56	7.22	7.33
II	10.14	9.42	6.24	8.19	8.06	6.13	6.09	7.65
III	10.22	9.48	6.12	5.97	6.60	7.10	6.12	6.91
IV	10.44	9.74	6.05	7.63	7.82	6.11	7.35	6.85
	Concentrate DM intake (Kg/day)							
I	12.52	13.56	8.94	10.46	10.70	9.53	9.40	10.06
II	13.60	13.07	9.04	10.63	10.72	9.64	8.77	10.31
III	14.54	10.93	8.51	8.48	8.66	10.33	9.06	9.60
IV	13.70	13.33	8.03	10.57	10.40	8.03	10.38	9.69
	Total DM intake (Kg/day)							
I	23.30	25.25	17.53	20.31	20.99	17.99	18.52	19.29
II	25.64	23.39	17.18	20.72	20.68	17.67	16.76	19.86
III	26.66	22.31	16.53	16.35	17.16	19.33	17.08	18.41
IV	26.04	24.97	15.98	20.10	20.12	16.04	19.63	18.44

CONTINUED . . . .

Appendix I - Table 7. CONTINUED

Periods/Cows No.	9	10	11	12	13	14	15	16
	Milk yield (Kg/day)							
I	38.9	41.0	25.2	28.2	29.1	25.7	24.5	26.5
II	38.3	40.3	25.7	27.0	28.0	25.7	20.9	24.7
III	37.4	35.7	24.7	23.6	26.8	26.3	20.0	23.8
IV	35.6	35.3	24.6	27.4	26.7	23.6	21.0	21.1
	Milk fat (%)							
I	4.22	3.65	3.88	3.17	4.45	4.08	3.20	2.70
II	3.90	3.44	3.77	3.42	3.75	3.77	2.49	3.67
III	4.09	3.41	3.68	2.33	3.25	3.55	2.99	3.88
IV	3.98	3.07	3.85	3.27	3.39	2.94	3.14	3.56
	Milk protein (%)							
I	3.36	3.51	3.71	3.58	3.51	3.50	3.50	3.60
II	3.60	3.72	3.88	3.72	3.60	3.47	3.65	3.82
III	3.68	3.85	3.85	3.95	3.68	3.53	3.78	3.83
IV	3.61	3.84	3.78	4.01	3.51	3.41	3.75	3.69

Appendix I - Table 8. Data on Digestibility and Nitrogen Balance Trial with Cows (Expt. II)

Period/ Cows No.	13	14	15	16
	DM digestibility (%)			
I	71.85	75.83	66.67	73.34
II	75.58	66.97	72.61	71.17
III	73.88	69.39	70.13	72.34
IV	65.82	74.16	66.03	75.34
	CP digestibility (%)			
I	65.07	66.53	65.07	72.03
II	71.92	64.58	71.92	68.34
III	74.50	66.00	74.50	67.34
IV	61.23	76.35	61.23	77.30
	ADF digestibility (%)			
I	46.77	51.64	33.13	46.41
II	50.54	26.95	43.32	40.67
III	41.87	40.89	44.98	43.70
IV	32.17	56.93	37.09	52.22
	Gross energy digestibility (%)			
I	73.40	70.59	66.31	66.55
II	76.16	73.56	72.42	66.23
III	66.88	69.23	76.44	74.49
IV	71.64	73.03	74.05	75.88

CONTINUED . . . .

Appendix I - Table 8. CONTINUED

Period/ Cows No.	13	14	15	16
	Nitrogen intake (g/day)			
I	422.4	396.8	366.4	377.6
II	481.6	384.0	324.8	414.4
III	355.2	400.0	401.6	396.8
IV	417.6	385.6	420.8	451.2
	Fecal N (% intake)			
I	25.8	33.5	34.9	28.0
II	22.6	35.4	28.1	31.7
III	22.5	34.0	25.5	32.7
IV	35.2	23.7	38.8	22.7
	Urine N (% intake)			
I	15.9	31.9	23.1	28.0
II	24.6	32.5	27.1	22.0
III	16.2	14.0	15.5	16.5
IV	22.2	26.6	18.3	22.0
	N balance (% intake)			
I	58.3	34.7	41.9	44.1
II	52.8	32.1	44.8	46.3
III	61.3	52.0	59.0	50.8
IV	42.5	49.8	43.0	55.3

Appendix II - Table 1. Switchback design and treatment sequence (Expt. III)

Periods/ Cow No.	Duration (days)	Treatment Sequence[1] Block I				Block II				Block III			
		1	2	3	4	5	6	7	8	9	10	11	14
Preliminary	14												
Period I													
Adjustment	7	A	B	C	D	A	B	C	D	A	B	C	D
Comparison	21												
Period II													
Adjustment	7	B	C	D	A	C	D	A	B	D	A	B	C
Comparison	21												
Period III													
Adjustment	7	A	B	C	D	A	B	C	D	A	B	C	D
Comparison	21												

[1] Treatment A, grass-legume silage + grain No. 1.
Treatment B, direct-cut fababean silage + grain No. 2.
Treatment C, wilted fababean silage + grain No. 2.
Treatment D, wilted fababean silage + grain No. 3.

Appendix II - Table 2. Data on some criteria measured on cows fed Experimental Diets (Expt. III)

Periods/ Cows No.	1	2	3	4	5	6	7	8	9	10	11	12
	Silage DM intake, Kg/day											
I	9.64	6.44	6.82	5.28	8.85	7.27	7.45	7.19	9.88	8.05	8.40	7.69
II	6.55	6.89	6.54	6.93	5.99	7.28	9.84	6.88	7.41	12.00	8.09	10.39
III	10.32	7.92	7.37	4.02	9.38	7.55	7.66	6.39	11.07	9.04	7.79	5.72
	Total DM intake, Kg/day											
I	18.84	17.03	15.63	13.95	17.55	19.01	18.23	18.79	18.95	20.62	20.50	19.12
II	16.36	16.47	17.27	16.76	15.19	19.36	17.88	17.38	19.86	21.51	19.29	18.90
III	19.44	20.09	17.11	11.99	17.85	19.26	17.88	17.36	20.89	22.49	18.12	16.20
	Milk yields, Kg/day											
I	27.77	17.67	21.10	24.61	27.20	24.07	26.63	26.23	24.43	32.73	27.50	27.42
II	19.97	16.10	19.00	21.90	24.30	21.63	23.03	22.90	23.03	20.57	23.97	24.27
III	21.60	15.60	19.20	20.07	22.63	15.73	22.83	21.07	20.23	22.27	21.87	22.07

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Appendix II - Table 2. CONTINUED

Periods/ Cows No.	1	2	3	4	5	6	7	8	9	10	11	12
	Milk butterfat, %											
I	3.11	3.83	3.74	4.08	3.51	2.84	3.30	3.63	4.45	3.59	3.64	3.58
II	3.39	3.71	4.09	3.46	3.26	2.85	3.32	3.72	3.70	3.47	3.59	3.74
III	3.55	3.66	3.42	3.66	3.47	3.02	3.42	3.53	3.98	3.34	3.58	3.91
	Milk protein, %											
I	3.12	3.35	3.76	2.93	2.78	2.97	3.00	3.09	3.29	2.90	3.32	3.26
II	3.39	3.61	4.11	3.59	3.20	3.36	3.08	3.23	3.89	3.24	3.48	3.33
III	3.54	3.80	4.45	3.71	3.48	3.47	3.41	3.64	4.19	3.66	3.62	3.86
	Milk SNF, %											
I	8.28	8.73	9.12	8.76	8.31	7.86	8.75	8.64	8.82	8.28	8.86	8.65
II	8.22	8.68	8.76	8.66	8.18	8.28	8.20	8.27	8.62	8.53	8.29	8.29
III	8.26	8.80	9.02	8.65	8.11	7.88	8.55	8.36	8.69	8.29	8.50	8.49

Appendix II - Table 3. Replicated Latin Square Design and Treatment Sequence (Expt. IV)

Periods/ Cows No.	Duration (days)	Treatment Sequence[1] Square I				Square II			
		13	14	15	16	17	18	19	20
Preliminary	14								
Period I									
Adjustment	14	A	B	C	D	A	B	C	D
Comparison	14								
Period II									
Adjustment	14	C	D	A	B	B	D	A	C
Comparison	14								
Period III									
Adjustment	14	D	C	B	A	C	A	D	B
Comparison	14								
Period IV									
Adjustment	14	B	A	D	C	D	C	B	A
Comparison	14								

[1]Treatment A, Untreated fababean silage + concentrate mix.
Treatment B, Untreated fababean silage + concentrate mix + encapsulated methionine.
Treatment C, Formaldehyde-treated fababean + concentrate mix.
Treatment D, Formaldehyde-treated fababean + concentrate mix + encapsulated methionine.

Appendix II - Table 4. Data on some criteria measured on Cows Fed Experimental Diets (Expt. IV)

Periods/Cows No.	13	14	15	16	17	18	19	20
	Silage DM intake, Kg/day							
I	8.12	7.04	7.40	7.50	8.62	8.82	6.32	9.50
II	7.54	8.52	7.42	9.16	10.12	8.42	8.48	9.21
III	8.72	8.52	8.54	8.24	9.14	9.51	7.38	9.52
IV	8.82	9.02	7.18	7.59	8.90	8.18	8.14	10.33
	Grain DM intake, Kg/day							
I	9.92	9.32	10.54	10.13	10.68	11.16	10.14	12.94
II	11.08	12.02	10.02	11.39	12.72	12.51	11.16	12.76
III	12.16	11.44	10.34	10.04	12.36	11.50	9.65	11.17
IV	10.82	11.01	9.25	9.91	11.25	10.66	9.99	12.22
	Milk yield, Kg/day							
I	28.2	27.2	22.9	27.1	28.2	22.4	21.8	22.6
II	25.4	35.0	22.2	27.0	28.8	22.4	23.4	21.0
III	26.0	29.4	21.1	23.8	26.4	22.6	23.0	19.8
IV	24.0	26.8	19.4	20.0	24.6	21.2	20.4	18.2

CONTINUED . . . .

Appendix II - Table 4. CONTINUED

Periods/Cows No.	13	14	15	16	17	18	19	20
	Milk butterfat, %							
I	3.57	3.12	3.56	3.40	3.72	4.29	3.68	3.53
II	4.05	3.36	4.08	3.42	3.68	4.37	3.61	3.78
III	3.99	3.23	4.00	3.43	3.93	4.26	3.43	4.12
IV	4.29	3.65	3.98	3.42	4.24	4.37	3.30	4.00
	Milk protein, %							
I	3.45	3.15	3.48	2.96	3.26	3.69	3.49	3.58
II	3.46	3.42	3.56	3.46	3.77	3.87	3.47	3.75
III	3.36	3.18	3.46	3.06	3.64	3.58	3.35	3.60
IV	3.60	3.31	3.48	3.07	3.62	3.62	3.27	3.67
	Milk SNF, %							
I	8.41	8.49	8.62	7.94	8.32	8.71	8.30	8.07
II	8.73	8.68	8.56	8.34	8.70	8.87	8.50	8.28
III	8.55	8.56	8.89	8.30	8.46	8.98	8.52	8.44
IV	8.63	8.34	8.30	7.81	8.21	8.74	8.06	8.20

Appendix II - Table 5. Data on Digestibility and Nitrogen Balance Trial with Cows Fed Experimental Diets (Expt. IV)

Period/ Cows No.	17	18	19	20
	DM digestibility, %			
I	72.35	60.63	60.07	56.67
II	60.47	60.86	66.43	60.21
III	66.82	66.67	65.92	65.43
IV	68.54	63.13	65.12	67.74
	CP digestibility, %			
I	71.72	62.96	59.17	55.36
II	62.08	60.63	68.83	62.41
III	70.78	65.89	70.83	67.22
IV	70.61	65.22	67.83	71.44
	ADF digestibility, %			
I	49.21	30.99	24.24	23.55
II	30.59	23.48	40.24	26.34
III	41.81	41.67	31.91	41.90
IV	40.95	28.26	33.45	39.25
	Gross energy digestibility, %			
I	70.72	58.45	58.58	55.09
II	58.86	58.69	69.83	58.56
III	66.07	65.45	67.05	64.39
IV	66.81	60.91	64.40	67.65

CONTINUED . . . .

Appendix II - Table 5. CONTINUED

Period/ Cows No.	17	18	19	20
	Nitrogen intake, g/day			
I	559.7	611.9	451.5	549.3
II	666.8	685.5	565.0	678.8
III	555.9	616.9	491.0	586.3
IV	597.1	562.1	496.5	669.1
	Fecal N (% intake)			
I	28.28	37.04	40.83	44.64
II	37.92	39.37	31.17	37.59
III	29.22	34.11	29.17	32.78
IV	29.39	34.78	32.17	28.56
	Urine N (% intake)			
I	31.70	34.68	36.79	42.85
II	39.80	28.71	36.60	43.53
III	65.13	43.49	53.12	47.77
IV	36.74	39.91	43.36	38.10
	Nitrogen balance (% intake)			
I	40.02	28.28	22.38	12.51
II	22.28	31.92	32.23	18.88
III	5.48	22.40	17.71	19.45
IV	33.87	25.31	24.47	33.34